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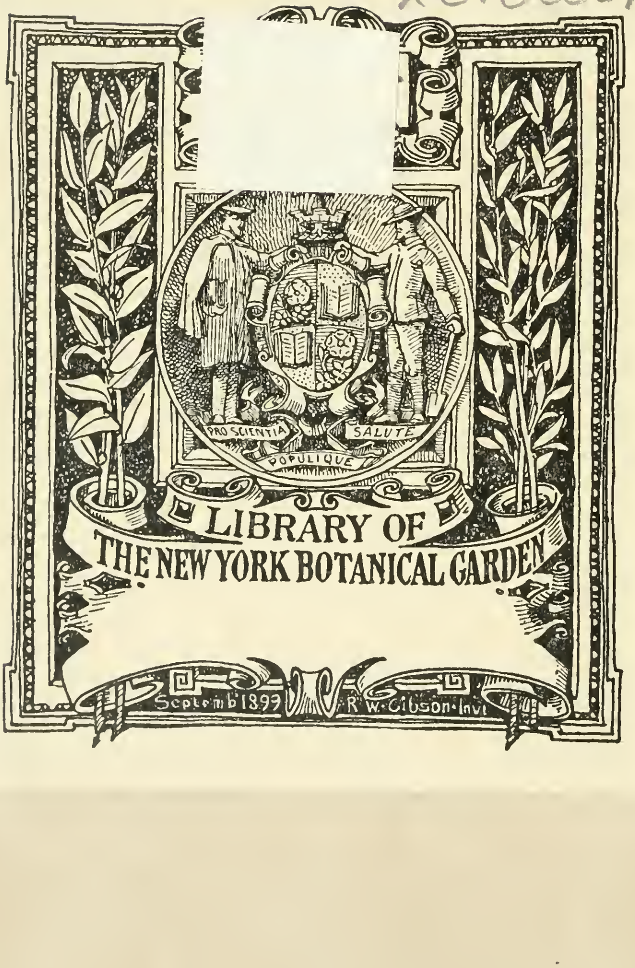
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SPERMATOGENESIS, OOGENESIS, AND FERTILIZATION IN NEPHRODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 107

(WITH PLATES VI-VIII)

SHIGEO YAMANOUCHI

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Historical review

The pteridophytes have been the source of important contributions to our knowledge of sperms in the plant kingdom, and investigations upon spermatogenesis in pteridophytes are already numerous. Historically, however, the motile sperm which first attracted attention was that of the algae, and among them especially the sperm of Chara.

From the early studies of spermatogenesis in Chara it was generally believed by NÄGELI (69), METTENIUS (62), HOFMEISTER (38), STRASBURGER (83), and SACHS (74) that the nucleus, before the formation of the sperm, dissolves into cytoplasm to form a homogeneous slimy protoplast from which the body of the sperm is organized. HOFMEISTER, STRASBURGER, and SACHS held a similar view concerning spermatogenesis in some forms of bryophytes and pteridophytes.

The first definite statement that the nucleus does not dissolve before the formation of the sperm was made by SCHMITZ (77) from a study of Chara and a number of mosses. He writes that the body of the sperm is produced by a direct change of form of the nucleus, whose peripheral layer becomes thickened to form a spirally coiled band, while the inner part becomes loose and forms a vesicle. The surface layer of the anterior end of the sperm, bearing cilia, consists of the cytoplasm which envelops that part of the nucleus.

JUN 13 1921

Most of the papers concerning spermatogenesis in pteridophytes were published after the appearance of SCHMITZ'S study of Chara, by such investigators as CARNOY (**16**, in Hymenophyllum), BELAJEFF (**1**, in Isoetes; **2**, **4**, in Gymnogramme and Equisetum), ZACHARIAS (**102**, in Pteris), CAMPBELL (**12**, in Onoclea, Asplenium, Gymnogramme, Adiantum, Alsophila, and Ceratopteris; **13**, in Pilularia; **14**, in Osmunda), BUCHTIEN (**10**, in Equisetum), LECLERC DU SABLON (**61**, in Cheilanthes), GUIGNARD (**31**, **32**, in Angiopteris, Pilularia, and Equisetum), SCHOTTLANDER (**78**, in Gymnogramme), and STRASBURGER (**85**, in Marsilia).

These studies begin with the structure of spermatids and are chiefly devoted to details of the events which occur within the spermatids during their transformation into sperms. Since the forms investigated were different, it is natural that the results should diverge in some particulars; but so far as the constituents of the mature sperm are concerned, the views fall into two categories. One group of authors, as ZACHARIAS, LECLERC DU SABLON, and BELAJEFF, believes that the spiral body of the sperm consists of a nucleus always enveloped by a layer of cytoplasm, which becomes the main constituent of the anterior coiled part of the body where cilia are produced; nuclear material does not extend to the anterior part. The other authors mentioned above hold that the nucleus is the main constituent of the body of the sperm, its anterior coiled part being enveloped with cytoplasm on which cilia are borne. Most observers of both groups agree that the cilia are produced from the cytoplasm, and that the vesicle (*Blase*) which is attached at the posterior end of the sperm is cytoplasmic in nature.

Most of the investigation of the origin and nature of the cilia-bearing organs of the sperms of pteridophytes has been done since the discovery of motile sperms and cilia-bearing structure in gymnosperms was made by IKENO (**40**, **41**, **42**, **43**), HIRASÉ (**36**, **37**), and WEBBER (**94**, **95**). The first paper that dealt with the details of the origin of cilia-bearing structures in the sperms of pteridophytes was that of BELAJEFF. Following his three preliminary notes (**3**, **4**, **5**), he published in 1898 an account (**6**) of spermatogenesis in Gymnogramme and Equisetum. Two deeply staining bodies (which he called *Nebenkern*) appeared on opposite sides of the nucleus previous to the final mitosis in the antheridium which differentiates the spermatids. At this final division each spermatid receives a blepharoplast which lies close beside the nucleus. The blepharoplast begins to elongate and its elongation is followed by that of the nucleus, so that both structures form two parallel bands which take a spiral form.

SHAW'S paper (**80**) on Onoclea and Marsilia appeared almost simultaneously with the foregoing contribution of BELAJEFF. He investigated the cell divisions preceding the formation of the spermatid in Marsilia and found two small bodies, which he called "blepharoplastoids," first in the daughter nuclei after the mitosis which differentiates the spermatid grandmother cell. There is one blepharoplastoid, therefore, for each nucleus of the grandmother cell of the spermatid. This blepharoplastoid divides, but the halves remain close together and pass to one side of the cell. With the next mitosis which occurs in the grandmother cell two new blepharoplasts appear at the pole of the spindle, and they accompany each daughter nucleus after this mitosis into the spermatid mother cell. Then each divides, and after the division of the spermatid mother cell, a blepharoplast is included in each spermatid.

The next year BELAJEFF (**7**) studied Gymnogramme and also the same species of Marsilia which SHAW had investigated. He found centrosome-like bodies at the poles of spindles in various mitoses preceding the formation of the spermatids with their unquestioned blepharoplasts, and he also found the blepharoplasts at the poles of the spindle. From this he drew the conclusion that the blepharoplast of Marsilia holds the same relation to the pole of the spindle as does a centrosome.

THOM (**88**) in the same year observed a blepharoplast in the spermatids of Adiantum and Aspidium as a round body beside the nucleus. He states that the disappearance of nucleoli at the time of the appearance of the blepharoplast would suggest a relationship between them. According to his observation the modification of the blepharoplast into a band structure is considerably delayed after the transformation of the nucleus.

No contribution based upon the original investigation of spermatogenesis in pteridophytes has been published since THOM'S paper appeared. However, the problem concerning the nature of the blepharoplast and its relation to the centrosome, not only in pteridophytes but in various groups of plants, has been discussed by STRASBURGER (**87**, with a critical review of the works on this subject), DANGEARD (**21**, on algae), TIMBERLAKE (**89**, on algae), WEBBER (**96**, on Zamia), IKENO (**45**, on Marchantia; **46**), DAVIS (**24**), and some others.

Oogenesis in pteridophytes has not aroused as much interest as spermatogenesis. Oogenesis in Filicales has been observed in various forms by JANCZEWSKI (**49**), GOEBEL (**29**), BRITTON (**9**), CAMPBELL (**15**), and many others.

Morphological investigations of the processes of fertilization in pteridophytes have been published by STRASBURGER (**83**, on Pteris and Ceratopteris), HANSTEIN (**33**, on Marsilia)), CAMPBELL (**13**, on Pilularia; **14**, on Osmunda), SHAW (**79**, on Onoclea), and THOM (**88**, on Adiantum and Aspidium). A few points of cytological interest in their studies concerning fertilization in pteridophytes may be summarized as follows:

In his paper on Osmunda, CAMPBELL writes that in several instances, when the archegonium seemed about ready to open, an appearance was observed that looked very much like the formation of a true polar body, and was not to be compared with the ventral canal cell. He suggests that this structure, like the polar body, may be derived from the division of the nucleus of the egg, his reason for the suggestion being the apparent diminution of the nucleus of the mature egg at the time of the appearance of the structure, although actual division was not observed.

In Onoclea SHAW states that before the archegonium opens, the egg is depressed above, and the nucleus of the egg is flattened, due to the pressure caused by the swelling of the wall of the ventral canal cell. As soon as the archegonium opens and the disorganized contents of the neck canal cells are expelled, the egg becomes turgid. After the sperm has entered the egg at the receptive spot, the egg collapses, and he suggests that this prevents the penetration of more than one sperm. MOTTIER (**67**) expressed some doubt whether the collapsed appearance is really normal.

In Pteris STRASBURGER (**83**) observed the formation of a membrane around the fertilized egg within a very few minutes after the entrance of the sperm, while SHAW in his paper (**79**) on Onoclea found no evidence that a membrane of appreciable thickness was formed immediately after the entrance of sperm.

In his account of Adiantum and Aspidium, THOM finds that when the sperm reaches the nuclear membrane, the nuclear part of the sperm finds or breaks an opening into the egg nucleus and cytoplasmic envelope, while the blepharoplast and the cilia are left out in the cytoplasm of the egg. Whether blepharoplast and cilia are left out in the cytoplasm SHAW leaves in doubt.

That the egg nucleus during the entire process of fertilization is in the resting condition and that the sperm enters the egg nucleus before undergoing any change in form or visible structure, are points of agreement among these authors in their studies on Adiantum, Aspidium, Onoclea, and Osmunda. One peculiar case, different from the foregoing results, was reported for Pilularia by CAMPBELL (**13**), who finds that a sperm nucleus assumes a loose and more granular structure and rounds up before entering to unite with the nucleus of the egg.

No work has been published showing details of the behavior of chromosomes during spermatogenesis, oogenesis, and fertilization in pteridophytes, nor has the organization of the figure of first segmentation division ever been followed.

The present investigation of this subject, with a study of sporogenesis which was considered in my recent paper (**101**), was carried on to trace out the complete history of the chromosomes throughout the life-cycle of *Nephrodium molle* Desv. The former paper, together with the present investigation, may serve as an introduction to the study of apogamy in Nephrodium, which will appear in a subsequent paper.

Material and methods

Gametogenesis and fertilization were studied from prothallia raised from spores in the greenhouse of the Hull Botanical Laboratory during the five months beginning October 1906. The spores for this culture were collected from leaves of the same individuals of *Nephrodium molle* that furnished material for the study of sporogenesis.

Various fluids were used for killing and fixing. The best fixation for the present studies was obtained in the material killed and fixed in the following solution: 1 per cent. chromic acid 25^{cc}, 1 per cent. glacial acetic acid 15^{cc}, 1 per cent. osmic acid 10^{cc}, water 50^{cc}. The methods used after the material was thoroughly washed were similar to those given in the previous paper on sporogenesis (**101**).

Description

VEGETATIVE MITOSIS IN PROTHALLIA

Immediately after the germination of the spore, the nucleus in the prothallial cell is quite large, but shows a tendency to diminish gradually during the further development of the prothallia into many-celled stages. The events of the mitosis in any vegetative cell of a prothallium are not only repetitions of the same type, but they are similar to those of the sporophyte. For this reason no detailed account will be made at this time, but a few essential points deserve mention.

The chromatin reticulum of the resting nucleus of the prothallia, which has a ragged structure like that in a vegetative cell of the sporophyte, becomes transformed into a spirem that runs an irregular course within the nucleus, many tangled parts or loops coming into close contact with the membrane. The spirem then becomes rather smooth and uniform in thickness and very likely there is present a continuous spirem (*fig. 1*). A kinoplasmic cap of conical shape appears at each pole of the nucleus, consisting of a few fibers arranged along the lateral edges of the cone. With the dissolution of the nuclear membrane at the two poles, the fibers push in and attach themselves to the chromosomes which have just segmented from the spirem (*fig. 2*).

The points at which segmentation of the spirem into chromosomes may occur have a certain regularity in relation to location within the nucleus. Although it is impossible to give an accurate explanation of the mechanism at present, the fact that many free ends of the chromosomes, immediately after segmentation, are directed toward the poles may prove that the segmentation took place in bent points of loops. Longitudinal splitting of the chromosomes appeared after the completion of this segmentation.

Contemporary with the entire dissolution of the nuclear membrane, the chromosomes show a tendency to arrange themselves parallel to an axis passing between the two poles (*fig. 3*). The daughter halves of chromosomes either remain in contact side by side throughout their length, or diverge in the middle part, remaining attached only at the ends; but the latter case is of short duration and soon they become reunited in pairs. The chromosomes now shorten and assume an L-shape, and there is established an early condition of an equatorial plate in which the vertical arms of the L-shaped chromosomes are parallel and directed toward the pole, while the horizontal arms lie in the equatorial plane; the spindle threads are attached at the ends directed toward the periphery of the equator (*fig. 4*). A polar view in this stage shows that the horizontal arms hold almost a radial arrangement, while the vertical arms are visible only in the optical section of their ends (*fig. 5*). The shortening and thickening of the chromosomes advance farther until they become short, rod-shaped, and arranged in the equatorial plate.

The separation of daughter chromosomes begins at once (*fig. 6*). As a rule, it takes place similarly in each chromosome, so that the daughter chromosomes proceed toward the pole as a set (*fig. 7*). When the set of daughter chromosomes has reached the pole, they remain for a short time as regularly arranged and straight chromosomes (*fig. 8*), the polar view of which clearly shows that the number of the chromosomes is 66 or 64 (*fig. 9*). The daughter chromosomes grouped at the pole become drawn together tightly and vacuolization begins. When the nuclear membrane is formed, the chromatin structure of the daughter nucleus shows polarity (*fig. 10*). The formation of the cell plate is like that described for the vegetative mitosis of the sporophyte.

SPERMATOGENESIS

A detailed description of the mitoses which take place from the cutting-off of an antheridium initial from a superficial cell until the formation of a spermatid mother cell will be omitted. The number of chromosomes is always 66 or 64. The only point which seems noteworthy is that in the central or primary spermatogenous cell there were observed in the majority of cases threadlike structures imbedded in the cytoplasm, sometimes very densely crowded together near the cell wall. The length of these threads was various, and they were a little more slender than the spirem that is usually present in the mitoses of spermatogenous cells. No such structure was ever observed in the superficial cells or in the antheridium initial from which the central cell is derived, and consequently this structure may originate in the central cell. It does not seem to play any important rôle in the nuclear division. As successive mitoses proceed, it becomes more faintly stained, less conspicuous, and finally becomes impossible to differentiate by staining; but in rare cases it still remains, filling the whole cytoplasm up to the eight- or sixteen-cell stage of the spermatogenous cells. A structure of similar appearance was observed in the central cell of the archegonium (*figs. 41, 41a*).

The spermatid mother cell is characterized by the first appearance of blepharoplasts. In general, the spermatogenous cells, in spite of their small size compared with the vegetative cells of the prothallia, contain large nuclei. The cytoplasm seems generally destitute of plastids and has a very fine fibrillar structure, and the membrane is delicate. The mitoses which take place in the spermatogenous cells are conspicuous on account of the comparatively large amount of chromatin, but otherwise they resemble those of the vegetative cells.

In the telophase of the mitosis, previous to the formation of the spermatid mother cell, the groups of daughter chromosomes, having reached the poles, begin to be vacuolized. Detailed study of this stage shows that the formation of the membrane around the mass of vacuolized chromosomes, or young daughter nucleus, is a little delayed in the polar region, which appears concave from the side view (*fig. 11*). The nucleus when viewed from the pole shows that the chromatin material resulting from the vacuolized chromosomes is very scanty in the polar region and three or four nucleoli are always present (*figs. 12, 13*); such a structure of the nucleus may be regarded as a kind of polarity. If there could possibly be any migration of nucleoli from the interior of the nucleus to the cytoplasm, it would certainly occur in such a critical stage of the nucleus, but there was not observable any peculiar differentiation within the cytoplasm that might warrant the supposition.

As the young daughter nucleus grows in size and assumes a spherical form, the chromatin loses its peculiar arrangement and becomes transformed into the irregular ragged reticulum of the resting condition. Then there are observed by their differential staining two small bodies, the primordia of the blepharoplasts, lying within the cytoplasm at the opposite sides of the nucleus. The first differentiation of the blepharoplasts by stains is very sudden, and they are unexpectedly large, quite surprising in fact, for one would imagine that they should appear at first as very small granules of hardly distinguishable size. The blepharoplasts move toward the nucleus, and during mitosis they hold positions near the poles of the spindle, without any noticeable increase in size (*figs. 14–19*). Sometimes (not often) it is observed that the blepharoplasts lie just at the poles of the spindle, but evidently this does not seem to mean that the blepharoplasts function as centrosomes; instead, the blepharoplasts in this case may have happened to be caught accidentally in the focal centers of the spindle.

In the anaphase of this mitosis 66 or 64 chromosomes are always counted (*fig. 20*). After the telophase each daughter cell of the spermatid mother cell, or each spermatid, contains a single blepharoplast, which now lies very close to the concave side of the nucleus.

When the cell plate is just being completed, a new body appears near the nucleus of the spermatid far away from the blepharoplast (*fig. 21*). For the sake of convenience, I called this body a *Nebenkern* in the preliminary note. It should be understood that this term is used simply as a temporary designation, which must be replaced by a more accurate one. The *Nebenkern* in Nephrodium always appears in the location previously occupied by the central spindle of the last mitosis. This spindle has almost disappeared when the *Nebenkern* appears. Accordingly, it is not probable that the *Nebenkern* originates from the spindle.

The spermatids grow to a certain stage and become almost free from one another, so that the antheridium now contains eight to thirty-two polyhedral or spherical spermatids, with very delicate membranes. During the growth of the nucleus of the spermatids the nuclear reticulum becomes transformed into a ragged network, very rich in irregular chromatin clumps, but with scanty branched strand structures. One or two nucleoli are always present (*fig. 22*). The blepharoplast, a round granule by this time, becomes applied to the nuclear membrane, and the *Nebenkern*, also a round body a great deal smaller than the blepharoplast, lies in various locations within the cytoplasm.

Now the modification of the blepharoplast begins. While the spermatid does not grow further, the nucleus and the blepharoplast increase in size during this metamorphosis, and the space occupied by the cytoplasm consequently gradually diminishes. The blepharoplast, now round in shape, lies very close to the nucleus and elongates along its spherical surface (*figs. 23, 24*), so that the surface view of the blepharoplast at this time shows a rhomboidal outline (*fig. 23*) and its cross-section is crescent-shaped, the concave surface being in close contact with the nuclear membrane (*fig. 24*). The elongation of the blepharoplast now proceeds farther. *Fig. 26* shows the section of the blepharoplast at this stage, cut parallel with the axis of elongation. The cross-section of the blepharoplast is shown in *fig. 27*, and a part of the structure viewed from the surface is given in *fig. 25*.

Until this time the blepharoplast is alike at both ends, and no modification was ever observed in the structure of the nucleus; but soon after the blepharoplast has elongated into a semicircular band, a very complicated modification follows in both nucleus and blepharoplast. One end of the blepharoplast becomes wedge-shaped and loosely laid along the spherical surface of the nucleus (*fig. 28*), while the other end, which still remains pointed, comes into close contact with the nucleus and finally coalesces with it (*figs. 28, 29*). The elongating blepharoplast now takes a spiral direction and has a tendency to form a coiled ribbon-like structure.

While the foregoing modification is occurring in the blepharoplast, the nucleus undergoes a characteristic transformation of form. The distribution of the ragged chromatin clumps within the nucleus becomes irregular; at certain regions of the nuclear cavity they become grouped very densely; while in other parts the chromatin substance is scattered. The nuclear membrane, very delicate at this time, seems to be easily influenced by any change which occurs in the interior of the nucleus, so that the region where the chromatin clumps are densely crowded may protrude above the spherical surface of the nucleus, while the region with scanty chromatin material may form a depression or furrow. This unevenness in the form of the nucleus, brought about by the irregular aggregation of the ragged chromatin clumps in different regions of the interior, develops in such a direction that the nucleus becomes metamorphosed into a coiled structure.

When the nucleus has almost assumed the coiled form, one end of the coil takes on a round sausage-shape (*fig. 30*), which gradually passes into a band structure (*fig. 29*). This band becomes more and more narrow, with a gradual diminution of its width, but this diminution is compensated for by a coalescence with the tapering end of the band-shaped blepharoplast; so that now the tapering end of the blepharoplast runs with the nuclear band as far as the latter structure goes, and finally the blepharoplast ends in the wedge-shaped band. Then the outer surface of that part of the coiled band, which is composed of the blepharoplast and nucleus by their parallel coalescence, becomes covered by the blepharoplast, which grows extensively.

The structure of the coiled nuclear region becomes more and more compact, during this metamorphosis of the nucleus, by the gradual condensation and aggregation of clumps of the chromatin reticulum. Thus the general structure of the coiled sperm is organized within the spermatid. By this time, numerous cilia are observed growing on the surface of the coiled band of the blepharoplast (*fig. 31*). During this modification of the nucleus and blepharoplast, the *Nebenkern* is always present in a definite position close to the nucleus, and when the coiled form of the sperm is established, the *Nebenkern* is observed near the blunt end of the sausage-shaped portion without entering into the construction of the body of the sperm (*fig. 32*).

The mature sperm when observed within the spermatid consists of 2.75 coils, 1.5 of which are composed of the blepharoplast with the nuclear substance lining a certain distance, and the rest exclusively of the nucleus. When the spermatids are discharged from the antheridium the anterior part of the sperm, which consists of the blepharoplast with cilia, first protrudes from the membrane of the spermatid, the membrane being so delicate at this time that it looks like a thin film of cytoplasm. As soon as the sperm begins to swim freely, the cytoplasmic substance with the *Nebenkern* is attached to the posterior coil of the sperm as a vesicle (*fig. 33*). The number of coils in the swimming sperm varies, but an average in material quickly killed is observed to be 2.75 coils. Numerous cilia grow from the whole of the surface of the band-shaped blepharoplast, their length sometimes surpassing more than twice the length of the body of the sperm.

OOGENESIS

The nuclei in the cells of the cushion region are generally larger than those of the rest of the prothallium. At first, successive mitoses that occur in the region to become the cushion divide cells anticlinally, so that there result a number of narrow parallel cells in a single row. In one of these cells, the next mitosis is periclinal and gives rise to an archegonium initial (*fig. 34*). From the archegonium initial, by two successive mitoses, there are cut off a basal cell, a primary neck cell, and a central cell, or primary oogenous cell (*figs. 35, 36, 37*). Up to this differentiation of the central cell, the critical stage of each mitosis showed that the number of chromosomes is 64 or 66.

The central cell grows in size even after its nucleus begins to divide and becomes characteristically asymmetrical in form, due to the influence of the surrounding cells. The cytoplasm in the central cell consists of a fine fibrillar structure in general, which passes into an alveolar structure toward the periphery (*fig. 38*). There are observed in the central cell thread structures of various lengths, scattered throughout the whole cell, especially near the cell wall. Their staining capacity and general aspect recall similar structures which are observed in the central cell of the antheridium.

The resting nucleus in the central cell increases in size considerably (*fig. 38*). The nuclear reticulum consists of ragged chromatin material and three or more nucleoli are present. There now takes place a mitosis to divide the cell into neck-canal and ventral cells (*figs. 39–42*). The mitosis is typically vegetative; in the prophase the spirem is continuous and a single nucleolus is present, perhaps resulting from the union of the nucleoli in the resting stage. The appearance of longitudinal fissions in the chromosomes, their shortening and their arrangement in the equatorial plate, were exactly similar to those which were described in the vegetative mitoses of the prothallia. In the anaphase the two sets of daughter chromosomes show no difference in the amount of chromatin material (*figs. 41, 41a*), but after the telophase the two daughter nuclei become unequal in size (*fig. 42*). The inequality of these two sister nuclei has resulted neither from unequal distribution of the chromatin mass nor from the number of chromosomes, but is a matter of nucleo-cytoplasmic relation, i. e., on account of the position of the nucleus of the central cell, the cell plate being laid down so as to divide the cytoplasm into two unequal cells. The immense amount of food material in the ventral cell causes its nucleus to grow more rapidly than that of the neck canal cell. At this time these two nuclei contain 66 or 64 chromosomes.

Then follows one more mitosis (simultaneous or successive) in each of these cells. The mitosis which divides the neck canal cell into two daughter cells is typical vegetative (*figs. 43–45*). In the telophase, when the daughter nuclei are formed, a cell plate is laid down as usual, but it soon begins to disorganize and finally disappears entirely. The direction of this mitosis is various, being sometimes parallel to the long axis of the archegonium, sometimes perpendicular to it, and sometimes oblique.

The ventral cell sooner or later divides into a ventral canal cell and an egg cell (*figs. 46–48*). No peculiarity is observed in this mitosis except in the metaphase (*fig. 46*), when there is almost always present a single dark staining body near the spindle, which possibly may be a persistent nucleolus, but its origin was not traced. The peculiar thread structure of unknown substance which is differentiated in the central cell is also continuously observed in the ventral cell, without any visible connection with the mitotic figure. In the telophase, the two daughter nuclei show a marked difference in size and shape, perhaps due to a nucleo-cytoplasmic relation, similar to the case previously observed (*fig. 47*). Several nucleoli appear in the young daughter nucleoli (*fig. 48*).

The characteristic curvature of the nucleus of the egg cell begins at the telophase of this division, and there is laid down by this time a dome-shaped cell plate which is of longer duration than that between the neck canal cells. The egg nucleus grows to an immense size and is very irregular in outline, whereas the neck canal cell and the ventral canal cell collapse and become mucilaginous, together with their disorganizing nuclei.

FERTILIZATION

The egg cell when ready for fertilization lies in the bottom of the archegonial cavity. Some of the material resulting from the disorganization of canal cells remains in the neck and ventral region of the archegonium as a slimy substance, even after the wide opening of the neck admits free access of water. The dome-shaped cell wall which separated the egg cell from the ventral canal cell seems almost to disappear at the time of the disorganization of the ventral canal cell, so that the protoplast of the egg cell is exposed at the neck side to the dilute slimy substance, but this exposed surface retains its irregular dome-shape.

The cytoplasm of the egg cell has a fine fibrillar structure, very dense around the nucleus and vacuolate toward the periphery of the cell. The upper part of the nucleus becomes depressed, so that its concave side is directed toward the neck, and it presents a crescent form in the side view (*figs. 48, 49*). The chromatin material within the nucleus becomes transformed completely into a system of anastomosing and branching fine strands and small ragged clumps, characteristic of the resting condition. One, two, or more small nucleoli are present.

Several sperms enter the neck of the archegonium and reach the surface of the exposed protoplast of the egg cell at the so-called receptive spot (*fig. 50*). Normally one of these sperms penetrates the cytoplasm of the egg. The cytoplasmic layer which surrounds the egg nucleus at this time is rather shallow on the exposed surface in the direction of the neck, so that the sperm has to pass through only a shallow layer of the cytoplasm. When the sperm reaches the nucleus, the coil becomes shortened and the penetration of the sperm into the nucleus begins from the anterior region, followed gradually by the posterior region (*fig. 51*).

During the penetration of the sperm the chromatin reticulum of the egg nucleus persists as it was before, but a peculiar irregularity of the reticulum is observed to have occurred near the intruding sperm, which in penetrating has presumably caused a movement of the contents of the egg nucleus. After the sperm has completely penetrated into the egg nucleus, this disturbance of the homogenous arrangement of the chromatin reticulum is still observed so long as the sperm within the egg nucleus maintains its individual outline (*figs. 52a, b*).

It is also observed that there is present a clear region directly surrounding the body of the sperm within the egg nucleus, and this seems to be a transient stage preceding the disintegration of the body.

The disintegration takes place in the body of the sperm in the very situation it occupied within the egg nucleus. The chromatin material, which was aggregated into a compact and condensed structure up to this time, now begins to return into a loose anastomosed complex of numerous chromatin clumps and branched fine strands, similar to those which were observed during the metamorphosis of the nucleus to organize the sperm. Small new nucleoli make their appearance near or in the disintegrated region (*figs. 53a, b*). Whether these nucleoli result from the disintegrated blepharoplast or nuclear region of the sperm or from some different source was not determined. If the blepharoplast originated indirectly in the interior of the nucleus from nucleoli which might have escaped during the reconstruction of the nucleus of the spermatid mother cell in the last telophase (*fig. 11*), and by a temporary change of staining capacity may have escaped observation until they first become differentiated by stains as two bodies, it might be expected that it should now reappear or return again into nucleoli as it disintegrates. Thus close relationship between the blepharoplast and nucleolus might be established. However, this is entirely a speculative view, which lacks evidence sufficient to make the suggestion probable.

Disintegration of the body of the sperm proceeds still farther, the anastomosing chromatin material showing presently the ragged reticular structure characteristic of the resting condition (*figs. 54a, b*), and finally the chromatin material of the sperm nucleus becomes entirely anastomosed with that of the egg nucleus whose membrane now surrounds the chromatin of both egg and sperm (*fig. 55*).

The fusion nucleus in the resting condition contains two or more nucleoli, some of which have appeared at the time of disintegration of the sperm nucleus, while others have probably resulted from the union of nucleoli which already existed in the egg nucleus (*fig. 55*). Figures of the first segmentation division are obtained in material fixed a week after the entrance of the sperm had been observed. The spirem which is organized at the expense of the chromatin reticulum of the fusion nucleus has a continuous structure, without any indication of two chromatin constituents of maternal and paternal origin. Whether there exists one or two spirems was not determined, but even if there be present more than one spirem they are exactly similar in thickness (*fig. 56*). Then the same process occurs as was described in vegetative mitosis. On account of the large number of chromosomes, the best stage for an accurate counting is obtained in the late anaphase, in which two sets of daughter chromosomes are regularly arranged near the poles (*fig. 57*). The polar view of this stage showed 128 or 132, or in rare cases 130, chromosomes (*fig. 58*).

Mitoses that occur following this first segmentation division, during the development of the embryo, were traced up to the appearance of the first leaf. The process is essentially similar, and 128 or 132 chromosomes are invariably present.

Discussion of cytological phenomena

The origin of the blepharoplast.—There have been many contributions concerning the origin and structure of the blepharoplast, not only in sperms of gymnosperms and pteridophytes, but also in zoospores of thallophytes.

In Cycas (IKENO **41, 42, 43, 48**), Ginkgo (HIRASÉ **36, 37, 48**), and Zamia (WEBBER **94, 95, 96**), two blepharoplasts first are formed *de novo* in the cytoplasm of the body cell at some distance from the nucleus, and one of these blepharoplasts is included in each spermatid; accordingly the origin of the blepharoplast is cytoplasmic.

In Equisetum and Gymnogramme, BELAJEFF (**6**) observed the blepharoplasts as two deeply stained bodies on opposite sides of the nucleus previous to the final mitosis which differentiates the spermatid. In Marsilia SHAW (**80**) discovered two blepharoplastoids in the telophase of the mitosis which differentiates the grandmother cell of the spermatid. The blepharoplastoids disappear in the spermatid grandmother cell, in which two new blepharoplasts are formed at the poles of the spindle. BELAJEFF (**7**) examined the same form which SHAW had studied, and he found two centrosome-like bodies at the poles of the spindle of the mitosis which gives rise to the spermatid grandmother cell. These centrosome-like bodies are probably structures similar to the blepharoplastoids of SHAW; however, according to BELAJEFF'S accounts, these structures do not pass into the cytoplasm to disappear, but accompany each daughter nucleus in the telophase and in the prophase of the next mitosis which occurs in the spermatid grandmother cell. This centrosome-like body divides and the two resulting ones become situated at the poles of the spindle. The process is repeated until there is formed a spermatid in which this centrosome-like body remains and becomes the blepharoplast.

Thom's account (**88**) for Adiantum and Aspidium differs from those of the last two authors. In regard to the origin of the blepharoplast he states that the disappearance of nucleoli at the time of the appearance of the blepharoplast in the cytoplasm of the spermatid would suggest a relationship between them.

According to Ikeno's account (**45**) of Marchantia, a centrosome is formed within the interior of the nucleus of the entire series of spermatogenous cells up to the spermatid grandmother cell. This body moves to the nuclear membrane and is thrust out into the cytoplasm. It then lies outside of the nucleus and becomes the functioning centrosome, dividing to form two centrosomes that separate and occupy the poles of the spindle. After the mitosis that gives rise to the spermatids, the centrosome remains to become the blepharoplast of the sperm.

Regarding the origin and structure of the blepharoplast of thallophytes, Strasburger (**85, 87**) expressed the following view, chiefly based on his study of the zoospores of Vaucheria, Cladophora, and Oedogonium. In all of these forms, he states, the blepharoplast arises from the outer plasma membrane (*Hautschicht*), the nucleus lying close to the plasma membrane at the time when the blepharoplast is formed. Another view, based upon the zoospores of Hydrodictyon, was advanced by Timberlake (**89**), who found that after nuclear multiplication had ceased, segmentation proceeded until uninucleate masses of protoplasm become separated from one another as zoospores. Then a blepharoplast was formed, lying in contact with the plasma membrane. But before the appearance of the blepharoplast, he adds, a granule may sometimes be observed close to the nucleus and it is possible that this is the first appearance of the blepharoplast. Dangeard's study (**21**) on Polytoma also suggests some possible relationship between the blepharoplast and nucleus. In zoospores of this form, he finds that the blepharoplast is situated directly under the plasma membrane and that a delicate threadlike structure extends from it into the cytoplasm and sometimes ends at the side of the nucleus in a granule. Davis (**24 a**) traced the origin of the blepharoplast in Derbesia, in which the blepharoplast arises from the granules observed close to the nucleus. There is a time previous to the differentiation of the zoospore when the nucleus lies close to the cleavage furrow that finally separates the adjacent zoospore rudiments. Close to the nucleus he observed a number of granules which migrate toward the surface region of the protoplast of the zoospore and assume a ring-shaped arrangement. By the fusion of these granules the blepharoplast is established.

According to these results, excepting STRASBURGER'S on Vaucheria, Cladophora, and Oedogonium, the blepharoplast seems to originate in the interior of the cytoplasm (Cycas, Ginkgo, Zamia, Equisetum, Marsilia, Gymnogramme, Onoclea), sometimes from a position close to the nucleus (Adiantum, Aspidium, Hydrodictyon, Polytoma, Derbesia), and in still another case from the interior of the nucleus (Marchantia).

In Nephrodium, as has just been described, the mitoses which occur within the antheridium were all investigated, from the first spermatogenous cell until the final differentiation of the spermatid. The blepharoplasts were demonstrated by the differentiation of stains as two small deeply staining bodies appearing first within the cytoplasm of the spermatid mother cell. The nucleus of the spermatid mother cell is in the resting condition when these bodies appear, and during the mitosis which differentiates the spermatid the blepharoplasts are situated near the pole of the spindle. After the telophase one blepharoplast accompanies each daughter nucleus and there is established a spermatid with a single blepharoplast. Sometimes blepharoplasts appeared in spermatogenous cells of the four-celled stage of the antheridium, but it is certain that these four cells do not represent spermatogenous cells in an early stage previous to the formation of the spermatid mother cells; on the contrary, they are in this case real spermatid mother cells, the number of sperms produced in the antheridium then being only eight.

The relationship of the blepharoplast and the centrosome.—The centrosome in pteridophytes was figured first by HUMPHREY (**11**) and then by CALKINS (**39**) in the spore mother cells of such forms as Osmunda, Psilotum, Adiantum, and Pteris. One year after CALKINS' paper appeared, SHAW'S account (**80**) of Marsilia and Onoclea was published, according to which the blepharoplast has nothing to do with the mitosis as a center of the mechanism of nuclear division, but simply lies near the pole of the spindle. He does not regard the blepharoplast as a centrosome.

BELAJEFF'S conclusions (7) in reference to Marsilia oppose those of SHAW; for he believes that the blepharoplast always occupies the pole of the spindle and functions as a centrosome during mitosis. He claims that the blepharoplast in pteridophytes is homologous with the centrosome. He figures the division of the centrosome or blepharoplast in the spermatid mother cell previous to the division of the nucleus, and between the two separating daughter centrosomes or blepharoplasts a small central spindle is developed just as in certain animal cells.

IKENO (45) considers the blepharoplast of Marchantia to be actually a centrosome, as shown by its behavior during mitosis. He homologizes the *Nebenkörper*, the deeply staining body in the cytoplasm of the spermatid, with the blepharoplastoid of SHAW. Again he (46) discusses the homologous nature of the blepharoplast and centrosome in his paper entitled "Blepharoplasten im Pflanzenreich." The *Nebenkern* of BELAJEFF, he suggests, is homologous with the deeply staining body (the *Körperchen*) in the spermatid of animals. Last year IKENO (47) reasserted his belief that the blepharoplast is a centrosome. He thinks that the bodies now called blepharoplasts may not all be homologous structures, but he holds that the blepharoplasts of pteridophytes, gymnosperms, liverworts, and myxomycetes are of centrosome origin either ontogenetically or phylogenetically.

The foregoing accounts of IKENO confirm BELAJEFF'S view regarding the homology of centrosome and blepharoplast. STRASBURGER (87) wrote at length on this subject seven years ago, and does not accept BELAJEFF'S view. He emphasizes the kinoplasmic character of the blepharoplast, whether it be a differentiated region of the plasma (as he believes for the zoospores of Vaucheria, Cladophora, and Oedogonium), or a special development in the interior of the cytoplasm (pteridophytes and gymnosperms). He thinks that all kinoplasmic structures, be they centrosomes, centrospheres, or blepharoplasts, hold a very close physiological relation to the structure of the nucleolus, so that the blepharoplast might occupy the position of centrosome without being genetically connected with that structure.

In Nephrodium there is present no centrosome in the whole life-history, and the blepharoplasts which arise *de novo* in the cytoplasm of the spermatid mother cell do not play the part of a centrosome. Since centrosomes are not found in this form it is impossible of course to discuss any relationship between them. If any genetic relationship really exists between the two structures in the plant kingdom, evidence might be sought in some of the lower forms, such as Sphacelaria and some other species in Phaeophyceae, in which centrosomes are known and motile spores are produced.

Fusion of male and female nuclei.—The condition of sexual nuclei at the time of fertilization has been studied in numerous forms of different groups of plants.

Among thallophytes the fusion of gamete nuclei was observed in Closterium and Cosmarium (KLEBAHN **52**), Rhopalodia (KLEBAHN **54**), Cocconeis (KARSTEN **50**), Sphaeroplea (KLEBAHN **55**; GOLENKIN **30**), Oedogonium (KLEBAHN **52**), Vaucheria (OLTMANNS **70**; DAVIS **23**), Coleochaete (OLTMANNS **71**), Fucus (FARMER and WILLIAMS **25**, **26**; STRASBURGER **86**), Dictyota (WILLIAMS **97**), Batrachospermum (SCHMIDLE **76**; OSTERHOUT **72**), Nemalion (WOLFE **98**), Polysiphonia (YAMANOUCHI **100**), Basidiobolus (FAIRCHILD **27**), Albugo (WAGER **92**; STEVENS **81**, **82**; DAVIS **22**), Peronospora (WAGER **93**), Pythium (MIYAKE **63**; TROW **91**), Achyla (TROW **91**), Araiospora (KING **51**), Sphaerotheca (HARPER **34**), Pyronema (HARPER **35**). No matter whether a condition of heterogamy is established or not in these forms after the union of gametes or gametangia, the male nucleus or nuclei pass into the cytoplasm of the egg or oogonium and union takes place between the male and female nuclei in the resting condition.

Similar conditions regarding the union of the sexual nuclei in angiosperms were described by STRASBURGER (**84**) for Monotropa, by SCHAFFNER (**75**) for Sagittaria, by COULTER (**19**, **20**) for Lilium and Ranunculus, by MOTTIER (**66**, **67**) for Lilium, by LAND (**57**) for Silphium, and by others. MOTTIER gives the most detailed account of chromatin at the time of the union, and figures the gamete nuclei as uniting with their chromatin in the resting condition. In the forms mentioned above, the gamete nuclei, with a few exceptional cases, are of almost equal size at the time of union, and fusion of the chromatin of the two gamete nuclei takes place after the dissolution of the portions of the nuclear membranes which are in contact.

There are described among gymnosperms cases in which there is a marked difference in size between male and female nuclei, as Thuja (LAND **58**), Picea (MIYAKE **64**), Abies (MIYAKE **65**), Torreya (ROBERTSON **73**), Sequoia (LAWSON **59**), Cryptomeria (LAWSON **60**). In these species the sperm nucleus, being considerably smaller, becomes imbedded in a depression of the egg nucleus. However, the process of their union does not differ essentially from the cases observed in thallophytes and angiosperms, because the chromatin material of both gamete nuclei in resting condition fuses after the disappearance of the contiguous part of the nuclear membrane.

According to the accounts given by a number of authors there is still another case: in Larix (WOYCICKI **99**) and Taxodium (COKER **18**), the gamete nuclei which come into contact do not fuse, but the chromatin contents of both nuclei are kept in distinguishable maternal and paternal groups; while in Pinus (BLACKMAN **8**; CHAMBERLAIN **17**; FERGUSON **28**) and Tsuga (MURRILL **68**) the chromatin of sperm and egg nuclei remains separate, forming two spirems, and only after their segmentation into chromosomes are the two sets of structures brought together in the first cleavage spindle. In these cases there is never present a resting nucleus including both maternal and paternal chromatin within a common nuclear membrane.

In the case of Nephrodium, as already described, the sperm which entered into the egg nucleus was observed during a certain period without any visible change, entirely imbedded within the chromatin reticulum of the egg nucleus. The chromatin material which constituted the body of the sperm begins to disintegrate, and the final result is a reticular structure similar to that which we have noticed in the nucleus of the spermatid before the formation of the sperm. The reticular structures of both sperm and egg nuclei become anastomosed and mixed together entirely within the membrane of the egg nucleus. The spirem of the first segmentation division is organized from this reticulum as a continuous homogenous structure.

This process of disintegration of the body of the sperm within the egg in Nephrodium accords with THOM'S and SHAW'S accounts. The presence of the resting nucleus including both maternal and paternal chromatin within a common nuclear membrane as described in Nephrodium was also described by IKENO (**43, 44**) in Cycas and Ginkgo, these two being the only forms among Cycadales and Ginkgoales in which fertilization has been traced to the complete union of the two gamete nuclei.

There is only one paper that gives details of fertilization in a bryophyte, a contribution by KRUCH (**56**) on Riella. In this form he states that after the sperm enters the egg, a male nucleus is organized which increases in size until it is about equal to the egg nucleus. This differs from the condition in Nephrodium, for the sperm does not enter into the egg nucleus, but two nuclei fuse side by side with their chromosomes fully organized.

With respect to the cytoplasmic structures of the sperm, all investigations among cycads and in Ginkgo agree that they are left behind in the cytoplasm of the egg before the gamete nuclei unite. A similar condition is reported in both Adiantum and Aspidium. In Nephrodium the author cannot confirm the foregoing view, since cases were observed in which the blepharoplast was not left behind in the cytoplasm. However, such cases do not of course necessarily mean that this structure takes part in what may be regarded as an essential part of the process of fertilization.

Conclusion

Since the present investigation on spermatogenesis, oogenesis, and fertilization, together with the preceding study on sporogenesis, has been made to trace out the complete history of the chromosome in the normal life-cycle of Nephrodium as preparatory to the study of apogamy, the general conclusions will be given in the subsequent paper. However, the two principal points established by the present investigation may be stated here: (1) the counting of the number of chromosomes is possible in the gametophyte; (2) the number of the chromosomes in the gametophyte is constant (64 or 66).

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EXPLANATION OF PLATES VI–VIII

The figures were drawn with the aid of an Abbé camera lucida, under Zeiss apochromatic objective 1.5^{mm} N. A. 1.30, combined with compensating ocular 18, except *figs. 34–38, 41, 42, 46–50* drawn with compensating ocular 4, and *fig. 33* drawn with compensating ocular 18 under higher magnification obtained by extending the tube. The plates are reduced to one-half the original size.

PLATE VI

Mitosis in the vegetative cells of the prothallium

Fig. 1. Prophase; nucleus with a continuous spirem.

Fig. 2. The nucleus after segmentation of spirem into a number of chromosomes, most of them before the stage of longitudinal splitting; two kinoplasmic caps with a few fibers are present.

Fig. 3. The nucleus after disappearance of membrane; chromosomes which are split longitudinally show a tendency to become arranged parallel to the axis passing between the two poles.

Fig. 4. Late prophase; daughter halves of chromosomes are in close contact with each other; they have shortened to L forms and are regularly arranged in an equatorial plate; the vertical arms of the L's are directed to the poles, while the horizontal arms are lying in the plate.

Fig. 5. Polar view of the stage shown in *fig. 4;* the horizontal arms of the L's are in regularly radiating arrangement and the optical section of the vertical arms appear like dots.

Fig. 6. Early anaphase; daughter chromosomes separating in a group.

Fig. 7. Late anaphase; two sets of daughter chromosomes near the pole.

Fig. 8. Early telophase; daughter chromosomes regularly arranged at poles.

Fig. 9. Polar view of the stage shown in the previous figure; 64 chromosomes present.

Fig. 10. Telophase; two daughter nuclei and a cell plate formed; arrangement of chromatin substance in daughter nuclei showing polarity.

Spermatogenesis

Fig. 11. Telophase preceding formation of spermatid mother cells; nuclei of spermatid mother cells just organized; two nucleoli present in one of the nuclei.

Fig. 12. Condition of nucleus later than stage shown in *fig. 11;* chromatin material very scanty in polar region, where nucleoli are present.

Fig. 13. Still later stage than that in *fig. 12;* arrangement of chromatin material still showing polarity.

Fig. 14. More advanced stage than that shown in *fig. 13;* nucleus of spermatid mother cell almost in resting stage; no polarity in distribution of chromatin material; two blepharoplasts within cytoplasm on opposite sides of nucleus.

Fig. 15. Nucleus of spermatid mother cell with spirem; two kinoplasmic caps with a few fibers; blepharoplast near pole of fibers.

Fig. 16. Spirem segmented into chromosomes; nuclear membrane begins to disappear at poles of nucleus; blepharoplast near the pole.

Fig. 17. Late prophase; nuclear membrane has completely disappeared; chromosomes split longitudinally; blepharoplast near center of spindle.

Fig. 18. Anaphase; two sets of daughter chromosomes on the way toward the poles.

Fig. 19. Early telophase; daughter chromosomes grouped at pole with regular arrangement; blepharoplast retains same position as before.

FIG. 20. Polar view of stage shown in *fig. 19;* 66 chromosomes evident.

FIG. 21. Late telophase; spermatid mother cell has divided into two spermatids, each containing a single blepharoplast near concave side of young nucleus; *Nebenkern* near each nucleus.

FIG. 22. One of the spermatids after the cellular connection has become loose and the regular arrangement has been lost; nucleus larger; chromatin reticulum composed of ragged clumps and two nucleoli present; blepharoplast applied very close to nuclear membrane; *Nebenkern* a little apart from the membrane.

FIGS. 23, 24. Two different views of same stage; *fig. 23* shows surface of blepharoplast; *fig. 24* the cross-section; *Nebenkern* moved near nucleus after blepharoplast has begun to grow and elongate.

FIGS. 25–27. Three different views of the same stage; *fig. 25* shows part of surface view; *fig. 26* longitudinal section; *fig. 27* cross-section.

FIG. 28. Nucleus of spermatid has become spiral; free end of blepharoplast wedge-shaped, the other end gradually tapering and along one edge a tapering end of the nucleus runs from the opposite direction and coalesces.

FIG. 29. Stage of spermatid nucleus later than that in *fig. 28;* coiled form of nucleus farther advanced; one end of nucleus, consisting chiefly of the blepharoplast, clearly visible; the other end, terminating in a sausage-shaped structure, slightly recognizable in the center below the *Nebenkern*, which is drawn lightly.

FIG. 30. Stage similar to that shown in *fig. 29*, viewed from different direction; end of nucleus terminating in sausage-shaped structure visible.

FIG. 31. Almost mature sperm within a spermatid; whole structure of 2.75 coils, of which 1.2 coils are blepharoplast, the rest the nucleus that terminates in a blunt end; long cilia borne on outer surface of blepharoplast; *Nebenkern* not drawn.

FIG. 32. Optical section of side view of stage shown in *fig. 31; Nebenkern* larger and near blunt end of sperm.

FIG. 33. Sperm fixed in free swimming condition; anterior end consists of blepharoplast which gradually covers only outer surface, and is replaced by band structure of nuclear substance which finally ends in a sausage form; *Nebenkern* present within vesicle.

PLATE VII

Oogenesis

FIG. 34. Cross-section of part of prothallium where cushion region arises later; nucleus in telophase of mitosis which forms archegonium initial.

FIG. 34*a*. Nucleus shown in previous figure under higher magnification.

FIG. 35. Nucleus of archegonium initial in metaphase of mitosis which cuts off basal cell.

FIG. 35*a*. Nucleus shown in *fig. 35* under higher magnification.

FIG. 36. Division into central cell and primary neck cell.

FIG. 37. Central cell or primary oogenous cell.

Fig. 38. By successive divisions of primary neck cell and growth of central cell, a characteristic archegonial projection is formed; cytoplasm of central cell with peculiar thread structures.

Fig. 39. Nucleus of central cell in early prophase; spirem continuous; a single nucleolus.

Fig. 40. Early metaphase; chromosomes, daughter halves in pairs, have shortened.

Fig. 41. Anaphase; cell growth continuing during mitosis.

Fig. 41*a*. Same mitotic figure shown in *fig. 41* under higher magnification; two sets of daughter chromosomes almost similar in amount; thread structures in cytoplasm.

Fig. 42. Telophase; two daughter nuclei unequal in size; ventral nucleus considerably larger than nucleus of neck canal cell.

Fig. 43. Prophase of mitosis in a neck canal cell.

Fig. 44. Anaphase of same.

Fig. 45. Telophase; a cell plate laid down between two neck canal cell nuclei; cytoplasm with vacuoles of various sizes.

Fig. 46. Section of archegonium after formation of two neck canal cells; the two nuclei in contact, the cell plate having already disappeared; nucleus in ventral cell in metaphase.

Fig. 46*a*. Nucleus of ventral canal cell shown in *fig. 46* under higher magnification; deeply staining body near spindle.

Fig. 47. Section of archegonium; ventral cell nucleus in telophase; two nuclei of neck canal cell in contact.

Fig. 47*a*. Two daughter nuclei shown in *fig. 47* under higher magnification; size and shape quite different; both contain a number of nucleoli; dome-shaped cell plate laid down separating egg and ventral canal cell.

Fig. 48. Section of archegonium after completion of egg, ventral canal cell, and neck canal cells; nucleus of egg larger than others.

Fig. 49. Section of archegonium with open neck; canal cells becoming mucilaginous, with disorganizing nuclei; egg nucleus irregular; single nucleolus present, due to union of several nucleoli seen when nucleus was organized.

PLATE VIII

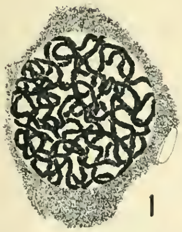
Fertilization

Fig. 50. Entrance of a number of sperms; many of them have already reached the egg.

Fig. 51. Union of sperm and egg; sperm partly within egg nucleus; cytoplasm of egg enveloping nucleus very shallow toward neck.

Figs. 52*a*, *b*. Two sections of same egg nucleus containing sperm, which has not changed; clear region surrounding sperm; upper side of figures toward neck.

Figs. 53*a*, *b*. Two sections of same egg nucleus containing sperm in later stage than that shown in previous figures; disintegration begun; compact body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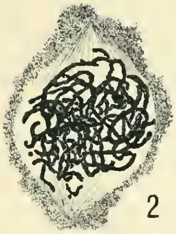
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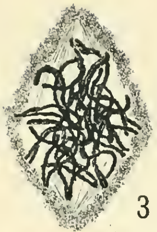
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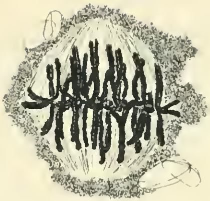
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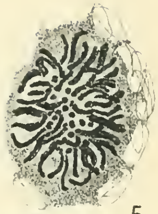
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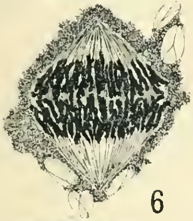
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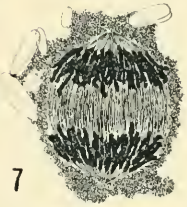
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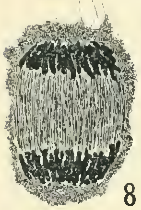
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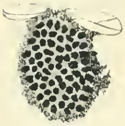
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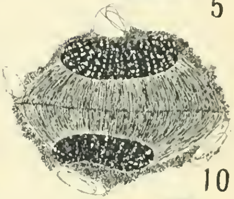
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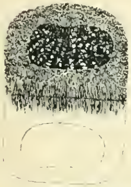
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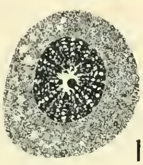
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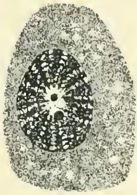
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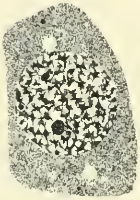
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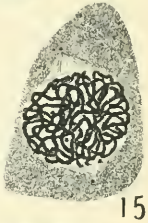
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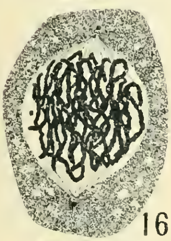
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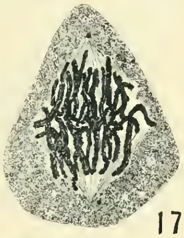
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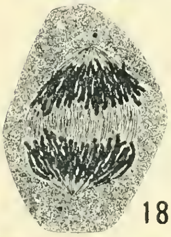
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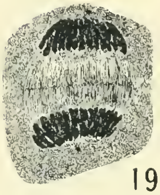
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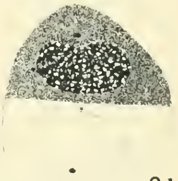
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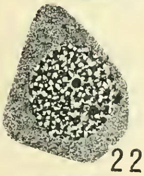
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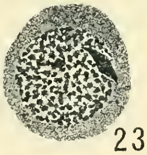
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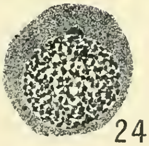
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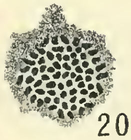
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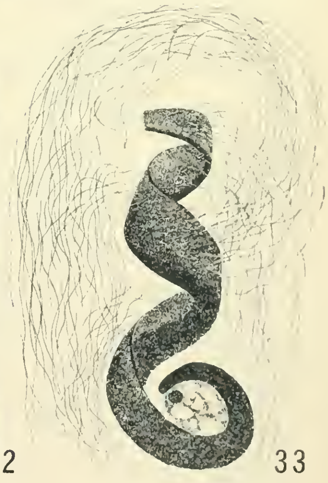
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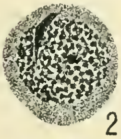
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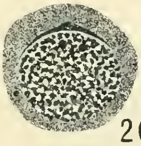
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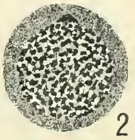
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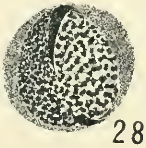
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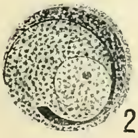
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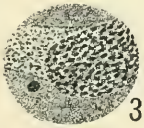
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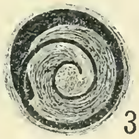
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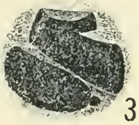
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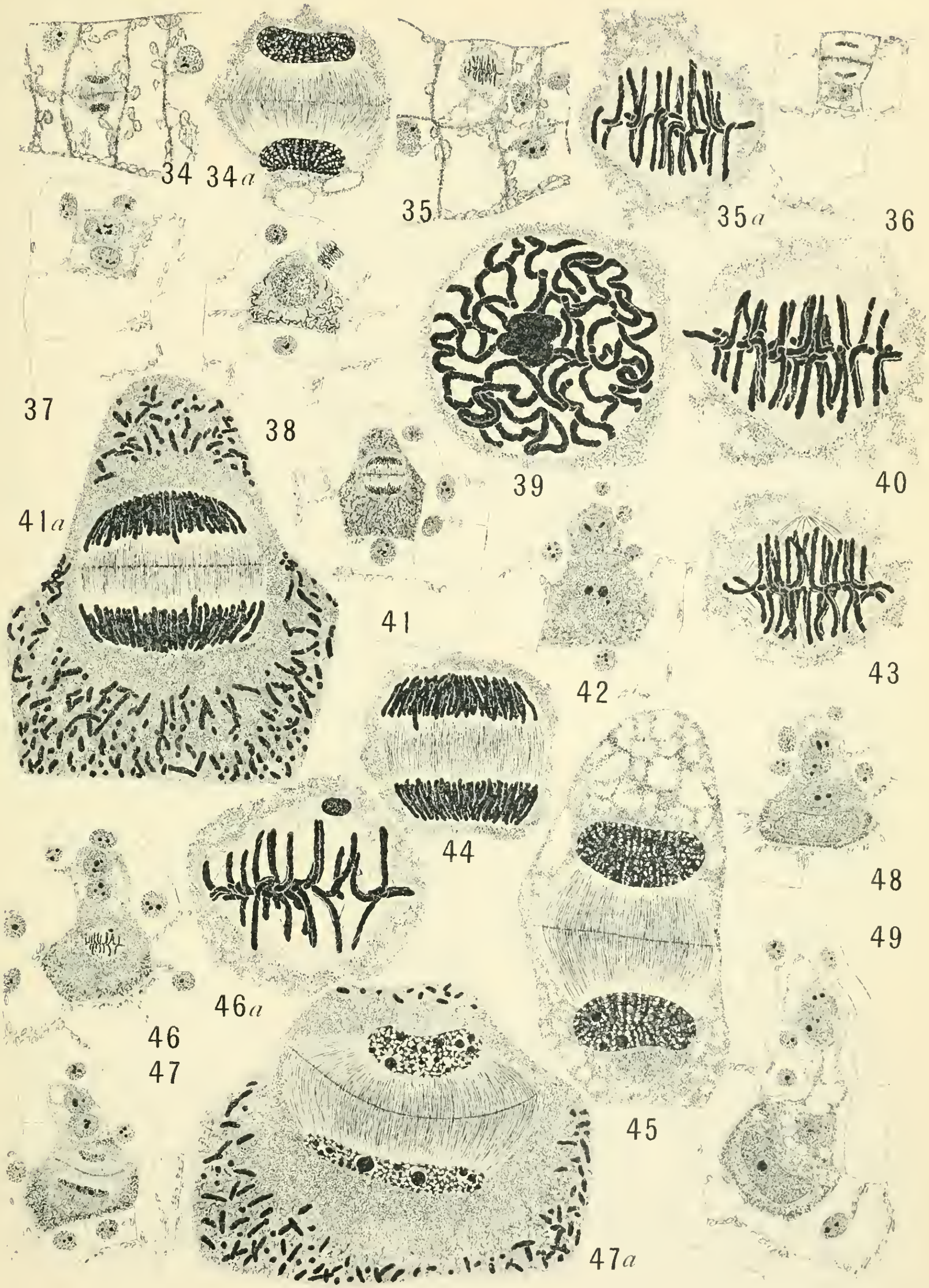
YAMANOUCHI on NEPHRODIUM



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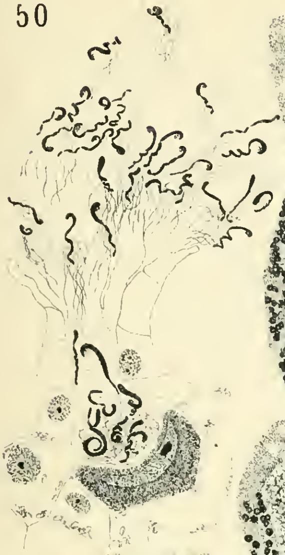
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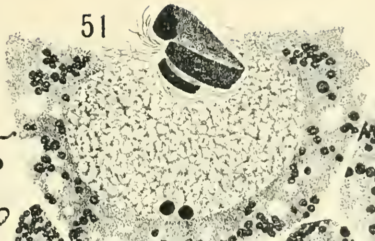
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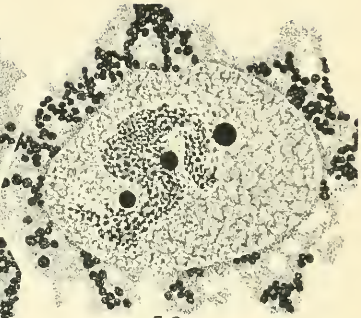
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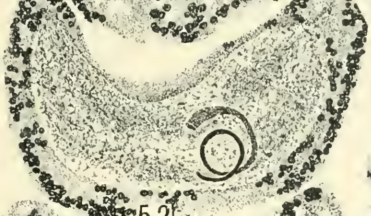
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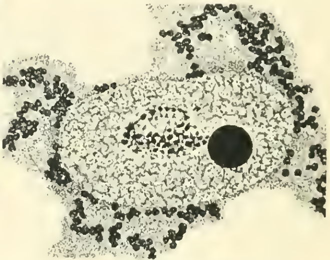
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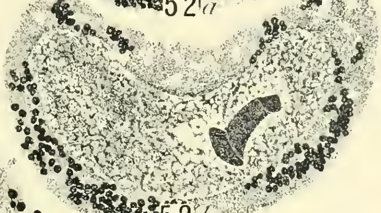
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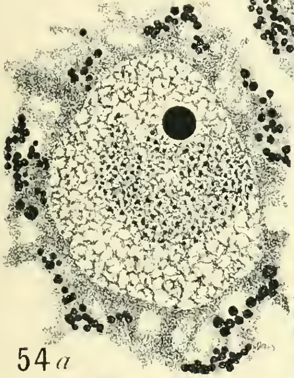
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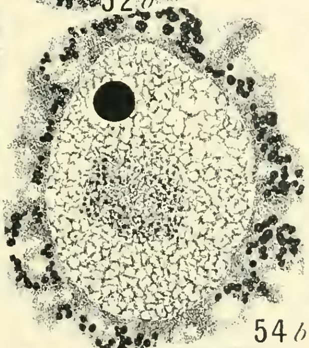
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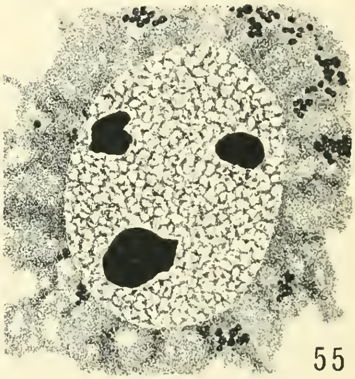
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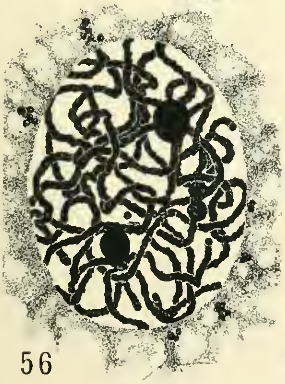
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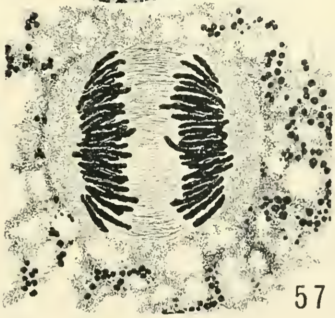
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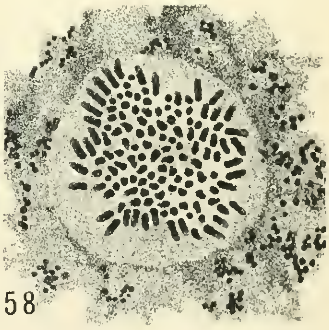
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YAMANOUCHI on NEPHRODIUM

of sperm loosened into complex structure of chromatin clumps and fine strands; three nucleoli in region of disintegrating sperm; upper side of figures toward neck.

FIGS. 54*a*, *b*. Two sections of egg nucleus containing a disintegrating sperm in stage later than in last figures; neck toward the left.

FIG. 55. Fusion nucleus after complete union of the two chromatin reticulums of sperm and egg; ragged reticular structure homogeneous throughout; three nucleoli present; upper side of figure toward neck.

FIG. 56. Spirem of first segmentation division of fusion nucleus shows a continuous structure.

FIG. 57. Anaphase of same mitosis; the two sets of chromosomes regularly arranged at the poles.

FIG. 58. Polar view of stage shown in *fig. 57;* 128 chromosomes present.

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THE CARBON ASSIMILATION OF PENICILLIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 108

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Since PASTEUR first showed that the lower fungi (yeasts in this case) could be grown on nutrient solutions containing organic substances other than those derived from plant and animal tissues, a large number of organic compounds have been examined with regard to their nutritive value for molds. It has been found that the most varied and widely different compounds are suitable in one case or another for supplying carbon and nitrogen to those organisms. All the fungi, however, cannot utilize the various compounds with equal facility. Thus, while Penicillium is almost omnivorous, thriving on alcohol, organic acids, sugars, and many other substances, *Mucor racemosus* and *M. javanicus*, according to WEHMER,[2] and yeast, according to LAURENT,[3] do not assimilate alcohol. In like manner, two very closely related compounds, such as the optically active modifications of tartaric acid, as well as chemical isomers without an asymmetric carbon atom,[4] may possess very different nutrient value.

The knowledge that a great number of organic compounds of the most varied structure could supply fungi with the necessary food and energy for all of their activities, soon led to attempts to establish some relation between the structure of organic compounds and their nutritive value. One of the first of these attempts was that of NÄGELI,[5] who came to the conclusion, as the result of the study of a large number of substances, that food value depended upon the specific linkage of

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[1] PASTEUR, L., Les corpuscules organisés qui existent dans l'atmosphère. Ann. Chim. et Phys. III. 64:5–110. 1862.

[2] WEHMER, C., Ueber das Verhalten der Mucor-Arten gegen verdünnten Alcohol. Ber. Deutsch. Bot. Gesells. 23:216, 217. 1905.

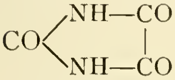
[3] LAURENT, E., Nutrition hydrocarbonée et formation de glycogène chez la levure de bière. Ann. Inst. Pasteur 3:113–125. 1889.

[4] BUCHNER, E., Notiz aus der Gährungschemie. Ber. Deutsch. Chem. Gesells. 25:1161–1163. 1892.

WEHMER, C., Beiträge zur Kenntnis einheimischer Pilze 87–104. 1903.

[5] NÄGELI, C., Ernährung der niederen Pilze durch Kohlenstoff- und Stickstoffverbindungen. Bot. Mitth. 3:395–485. 1881.

certain atomic groups. According to Nägeli, carbon is assimilated from the groups $=\mathrm{CH}_2$ and $\equiv\mathrm{CH}$, but in the latter case only when it occurs in a chain of two or more C-atoms to which H is bound. Benzoic acid is assimilated, but formic acid is not. On the other hand, carbon is not assimilated when it is directly united only with O or N but not with H. Accordingly, the CN group, oxalic acid, urea, and similar substances, are not suitable sources of carbon.

Subsequent researches showed that the conclusions of Nägeli were no longer tenable, since it was found that the molds were not restricted to such specific groupings as he supposed. Reinke[6] greatly extended the list of substances which could be assimilated, and showed that some of the particular groupings which Nägeli excepted could be utilized. Thus, for example, parabanic acid,  $\mathrm{CO}\left\langle\begin{array}{l}\mathrm{NH-CO}\\ \qquad\quad |\\ \mathrm{NH-CO}\end{array}\right.$ proved an efficient source of carbon for Penicillium. Diakonow[7] demonstrated that carbon from urea and from formic acid could also be utilized by this fungus.

All of these facts indicate that no general relation can at present be established between the atomic structure of a substance and its food value. The mechanism of assimilation probably differs with each individual case and is as much dependent upon the nature of the plant as upon the chemical reactions of the compounds used; for, as already stated, every substance that has nutritive value for one plant will not serve as a food for all plants.

It was with a view of gaining, if possible, some knowledge of the complex problem of the chemistry of the assimilation of some of the simpler compounds that the work reported here was undertaken. It was thought that by studying the effect of a number of related compounds on the growth of the mold fungi, and noting the variations caused by different stimulating agents, it would be possible to gain some knowledge of the probable chemical reactions by which nutrient substances combine with the constituents of the living cell.

This proved to be a most complex problem, whose solution is made

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[6] Reinke, J., Unters. Bot. Lab. Göttingen. (Rev. in Bot. Zeit. 41:551. 1883, and Just's Bot. Jahresb. 11:55. 1883.)

[7] Diakonow, N. W., Organische Substanz als Nährsubstanz. Ber. Deutsch. Bot. Gesells. 5:380–387. 1887.

more difficult on account of the narrow limits of experimentation to which living organisms can be subjected. Although no finally satisfactory conclusions have been reached, a few series of cultures have been worked out with great care. As I am for the present compelled by circumstances to discontinue the work, it is desirable to present the results thus far obtained, with a few final remarks on their possible significance.

METHODS

The form selected for this work was a strain of *Penicillium glaucum*. A number of other molds were tried, but were rejected because they did not thrive well under the conditions of the experiments. Penicillium, which has been used in innumerable physiological investigations, is especially suitable for this work on account of its omnivorous habit, and because many compounds have been studied with regard to their nutritive value for this fungus. The same strain of the mold was used throughout. The stock-cultures were always grown on sterilized bean-stems, so as to avoid any possible temporary influence of the substratum on the strain.

The culture medium used was a solution of the necessary inorganic salts, of the purest grades obtainable in the market. Except where specially noted, the culture fluid always contained the mineral salts in the same concentration, namely, 1^{gm} NH_4NO_3, 0.5^{gm} KH_2PO_4, and 0.25^{gm} $MgSO_4$, per 100^{cc}. Potassium chlorid was added in some cases, and the concentration of the magnesium salt was varied in others. These changes are noted in the respective tables. It was the purpose in this work to have the mineral solution alike in all the cultures in order to make the different series exactly comparable. This was carried out, except where special problems occurred which demanded a change from the standard.

As a source of carbon the following substances were used: alcohol (C_2H_5OH), potassium ethyl sulfate ($C_2H_5KSO_4$), ethyl nitrate ($C_2H_5NO_3$), ethyl acetate ($CH_3COOC_2H_5$), potassium acetate (CH_3COOK), and acetic acid (CH_3COOH). These compounds are all closely related, and have certain radicles in common. The combination of those radicles with various groups has a definite effect upon the mode of reaction of the compounds. By a comparative study of a large number of compounds of this nature with regard to their assimilation by the molds, it was deemed possible to throw some light on the mechanism of their assimilation. The simpler compounds are most suitable for studies of this nature; first, because their reactions are well understood, and second, because it is known that the complex compounds, such as polysaccharides, glucosides, and certainly in some cases even the hexoses, undergo decomposition before they are taken into the cell.[8] The solution of the mode of assimilation of the simpler compounds will therefore enable us to approach an interpretation of this process with the more complex compounds.

Cultures were made in 200^{cc} flasks, of the Erlenmeyer form, loosely stoppered with cotton to permit the free exchange of air. In each flask 50^{cc} of the culture medium were used. The mineral solution was first put into the flasks and sterilized for 20 minutes in flowing steam, after which the organic solution was added. The concentration of all the substances given is the final concentration after the addition of the organic solution, without allowing, however, for error caused by evaporation in the sterilizer, which averaged a little less than 0.5^{gm} per flask. In cases where acid was added the error caused by this addition was also not considered. These two errors compensated each other. Moreover, it was found that much greater differences than these produced no effect on the growth of the fungus, and a variation in the concentration is furthermore unavoidable on account of a slight evaporation in the incubator, which could not be prevented. The cultures were grown in an electrically regulated and electrically heated incubator, which permitted the maintenance of a constant temperature of 28° C. The duration of the cultures, except where noted, was 10 days.

Considerable effort was made to work out the best method for inoculating the flasks. On account of some observations of DUCLAUX,[9] indicating that some media which were suitable for nourishing grown mycelia were not suitable for the germination of spores, the spores used in the first cultures were germinated before inoculation. This method was extremely unsatisfactory, because the germinating spores could not be evenly distributed in the culture liquid. Moreover, it was found that spores germinated well in all the media used. Attempts to inoculate the flasks by means of the platinum wire also proved failures, because it was impossible to obtain an even distribution of the dry spores. The method finally adopted, which proved entirely satisfactory, was as follows: A stock-culture on beans, which was well covered with spores, was thoroughly shaken up with the liquid in which it was growing; the liquid was then poured on a screen of fine-meshed muslin in a funnel, and strained into a sterilized flask. This gave a liquid turbid with spores, most of which had been shaken apart and floated free in the liquid. Very little other material passed into the flask. For inoculation three drops of this liquid were dropped into each flask from a sterile pipette. This gave an abundance of spores, perhaps some thousands, which were uniformly distributed in the culture fluid.

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[8] PURIEWITSCH, K., Ueber die Spaltung der Glycoside durch die Schimmelpilze. Ber. Deutsch. Bot. Gesells. **16**:368–377. 1898.

BRUNSTEIN, A., Ueber Spaltungen von Glycosiden durch Schimmelpilze. Beih. Bot. Centralbl. **10**:1–50. 1901.

KOHNSTAMM, P., Amylolytische, glycosidspaltende, proteolytische und celluloselösende Fermente in holzebewohnenden Pilzen. *Ibid.* **10**:90–121.

[9] DUCLAUX, E., Sur la nutrition intracellulaire. Ann. Inst. Pasteur **3**:97–112. 1889.

The yield of dry material produced was used as an indicator of the effect of the food given. The yield was determined by adding 10^{cc} of a 10 per cent. solution of chemically pure hydrochloric acid to the culture to kill the growth and dissolve any precipitates that had been formed. After this the culture was filtered on a hard filter paper, from which the fungous material was washed into a tared Gooche crucible, and dried at 100–110° C. The variation in the temperature at which the yields were dried was not preventable, since no regulated drying oven was available. All the yields of each series were dried at the same time, however, so that they were subjected to the same conditions in drying. The different sets of each series are therefore comparable with each other.

TABLES AND EXPLANATIONS

In the following tables are given the results of the cultures, with the necessary explanations relating to each series. In the course of the work many hundreds of cultures were made before finally satisfactory details of manipulation were worked out, and for the purpose of determining the concentrations of the various substances that permitted the best growth of the mold. None of these preliminary series is included in this report, although the facts gained from them served in a general way to substantiate the results of the other cultures.

Alcohol.—One table, made for the purpose of determining how long the minimum quantity of alcohol used in some of the cultures could provide sufficient food for an increase in growth, may not be without interest and is here given as *table I.* The yield is given in milligrams in all cases.

TABLE I

Each flask received 0.46gm alcohol = concentration of 0.2 GM. per liter.

Time in days (24 h.)	1	2	3	4	5
1	0.0	0.1	.....	...	...
2	1.4	1.8	1.9	...	...
3	13	14	15	16	17
4	.....	.....	.....	...	...
5	38	39	43	53	58
6	59	61	65	79	92
7	80	91	95	104	110
8	118	119	120	122	124
9	118	121	124	128	128
10	110	113	114	115	120

All the cultures in each horizontal row are duplicates. An increase in weight took place up to the tenth day, after which there was a gradual loss, due to respiration.

TABLE II

Each flask received 0.69gm alcohol = concentration of 0.3 GM. per liter.

Number	I. No acid	II. $0.004n$HCl	III. $0.004nHNO_3$	IV. $0.004nH_2SO_4$	V. Check. No organic compounds
1	53	90	106	113	4
2	57	108	120	116	
3	86	114	125	131	
4	89	115	127	139	
5	93	118	144	141	

TABLE III

Each flask received 0.46gm alcohol = concentration 0.2 GM. per liter.

Number	I. No acid	II. $0.004n$HCl	III. $0.004nHNO_3$	IV. $0.004nH_2SO_4$
1	68	91	100	88
2	70	93	106	89
3	71	93	106	91
4	73	102	108	93
5	77	103	110	95

In about 200 preliminary cultures it was found that the addition of small quantities of acids such as HCl, HNO_3, and H_2SO_4 had a stimulating effect on the growth of Penicillium. This is only an instance of the general rule that poisonous substances act as stimulants when given in sufficiently dilute concentration. These two series were carried through with great care and uniformity, in order to get, if possible, a more accurate quantitative statement of the facts noted in the preliminary cultures. There is considerable individual difference in the cultures, as is noted by reading the figures in each column, the five cultures being duplicates of one another. Nevertheless, they are of the same general magnitude. The addition of mineral acids produces a decided stimulation of growth. The nitric acid in each case gave a greater stimulation than the hydrochloric. The sulfuric acid in one case (*table II*) gave a greater increase than hydrochloric, while in the other case (*table III*) its effect was about equal to that of the HCl. These observations agree with those made on the preliminary cultures. Many of the cultures seemed to show that nitric and sulfuric acids produced greater stimulation than hydrochloric acid. It was thought possible that HCl might begin to act as a poison at the concentration used, but a series of cultures (*table IV*) showed that the optimum for stimulation was much higher than 0.004*n*. Therefore HCl at that concentration cannot act as a poison, but is actually a milder stimulant than HNO_3, and perhaps also weaker than H_2SO_4. In addition to the usual mineral nutrients, the cultures in *tables II*, *III*, and *IV* were given 0.25^{gm} KCl per 100^{cc} of culture fluid, to avoid the introduction of a new ion, Cl, in the cultures to which HCl was added. None of the alcohol cultures produced spores.

TABLE IV

Each flask received 0.69^{gm} alcohol = concentration 0.3 GM. per liter.

Number	Concentration of acid								
	1 0.000	2 0.002*n*	3 0.004*n*	4 0.006*n*	5 0.008*n*	6 0.012*n*	7 0.016*n*	8 0.024*n*	9 Check
1	208	172	146	266	183	193	236	163	3.6
2	209	227	185	268	255	239	239	171	3.6
3	209	232	195	284	257	246	240	173	
4	214	239	266	285	260	255	246	181	
5	224	242	280	295	273	255	257	181	

Esters of alcohol with mineral acids.—The cultures with esters of alcohol with mineral acids gave the results expressed in the following tables. The actual quantity as well as the concentration of the organic substances in each flask are given in *tables V* and *VII* in the horizontal lines, and in *tables VI* and *VIII* at the head of the columns giving the yields for each set.

TABLE V

Numbers	Amount of $C_2H_5KSO_4$	Yield
1–10.......	0.82^{gm} (=0.1 GM. per l.)	none
11–20.......	1.64^{gm} (=0.2 GM. per l.)	none

TABLE VI

Number	0.82^{gm} $C_2H_5KSO_4$ (=0.1 GM. per l.) 0.69^{gm} C_2H_5OH (=0.3 GM. per l.)	0.82^{gm} $C_2H_5KSO_4$	0.41^{gm} $C_2H_5KSO_4$ (=0.05 GM. per l.) 0.69^{gm} C_2H_5OH	0.41^{gm} $C_2H_5KSO_4$	Check 0.69^{gm} C_2H_5OH
1.........	152*	None	200	None	193
2.........	184	None	210	None	
3.........	207	None	214	None	
4.........	219	None	228	None	
5.........	219	None	238	None	

* Contaminated.

TABLE VII

Numbers	$C_2H_5NO_3$*	Yield
1–10...............	0.46^{gm}	None
11–20...............	0.91	None

* The concentration of the ethyl nitrate is not stated, since only a part of the amounts given goes into solution in 50^{cc} of the culture fluid.

TABLE VIII

Number	0.23^{gm} $C_2H_5NO_3$ 0.69^{gm} C_2H_5OH (=0.3 GM. per l.)	0.46^{gm} $C_2H_5NO_3$ 0.69^{gm} C_2H_5OH	0.69^{gm} C_2H_5OH		Check. No organic matter
1............	103	52	68	117	2
2............	106	56	94	118	
3............	112	65	112	141	
4............	115	72	113	143	
5............	126	89	116	146	

The cultures of potassium ethyl sulfate (*table V*) had a barely visible film on the surface, similar to cultures to which no organic matter had been added. Microscopic examination showed that practically all the spores had germinated and had produced germ tubes 50 to 100 μ long. This is clear proof that the potassium ethyl sulfate is not poisonous in the concentrations used, but also that it is not a suitable source of carbon. This is more fully brought out in *table VI*. The cultures with potassium ethyl sulfate behaved as before, while those with sulfate to which alcohol was added gave a yield comparable with that obtained when alcohol alone was given. These cultures, like the alcohol cultures, remained pure white and produced no spores.

Ethyl nitrate in various concentrations ranging from 0.46^{gm} per 50^{cc} to 0.91^{gm} per 50^{cc} proved absolutely valueless as a source of carbon, although like potassium ethyl sulfate it did not inhibit germination at these concentrations. In a series in which the ethyl nitrate was given as the sole source of carbon and nitrogen, no growth took place. Neither is the fungus able to break up the nitrate by means of energy derived from alcohol, as *table VIII* shows, for the addition of ethyl nitrate failed to produce an increase of yield over pure alcohol. On the contrary, the higher concentration depressed the yield, showing that ethyl nitrate is mildly toxic. This is also shown by the fact that spores in cultures with ethyl nitrate germinated two to three days later than the alcohol cultures. The depression of yield may be due partly to the delay in germination, and partly to the toxic effect of the nitrate. The cultures produced no spores.

Ethyl acetate.—While the esters of alcohol with mineral acids proved valueless as a source of carbon for Penicillium, the ethyl ester of acetic acid in dilute solution is an efficient source of carbon; but in stronger solutions it becomes a poison, as appears from *tables IX* and *X*. The mineral solution used here was of the same composition as in the alcohol cultures.

TABLE IX

Each flask received 0.22^{gm} $CH_3COOC_2H_5 = 0.05$ GM. per liter.

Number	No mineral acid	$0.004nHNO_3$	$0.004nH_2SO_4$	$0.004nHCl$
1.	13	12	10	11
2.	14	12	11	11
3.	14	12	12	11
4.	14	12	12	12
5.	15	12	12	13

TABLE X

No.	I 0.88^{gm} $CH_3COOC_2H_5$ (=0.2 GM. per l.) 0.69^{gm} C_2H_5OH (=0.3 GM. per l.	II 0.88^{gm} $CH_3COOC_2H_5$	III 0.44^{gm} $CH_3COOC_2H_5$ (=0.1 GM. per l. 0.69^{gm} C_2H_5OH	IV 0.44^{gm} $CH_3COOC_2H_5$
1....	None	None	59	19
2....	None	None	74	20
3....	None	None	79	21
4....	None	None	89	22
5....	None	None	90	22

Ethyl acetate is easily soluble in water and forms a suitable source of carbon for fungi. It is poisonous, however, in comparatively low concentrations. The fungi in this medium grew for a time and then ceased growing, owing to the accumulation of acetic acid in the cultures. The addition of mineral acids had no very evident effect on the growth of the mold, but seemed to depress rather than increase the yield. It is important to note that the cultures containing alcohol in addition to ethyl acetate (*table X*, *col. III*) gave a yield about equal to that produced by alcohol alone (*table II*, *col. I*). It is probable that the alcohol, which is the better food, is largely responsible for this growth, and that the acetate was left largely intact. If it had been consumed in the usual way, the accumulation of acetic acid would have stopped the growth of the cultures. The cultures containing alcohol failed to fruit, while those containing only ethyl acetate fruited abundantly. The spores from sets I and II, *table X*, were killed, as no growth appeared when they were transferred to bean-stems.

Acetic acid.—Acetic acid in its free state forms an efficient source of carbon, but is so highly toxic that it must be used in extremely dilute solutions. Nevertheless, two series of cultures placed the fact of its assimilation beyond doubt. These are given here.

TABLE XI

Acetic acid in each flask 0.012^{gm} ($=0.004n$).

Number	No mineral acid	$0.004nH_2SO_4$	$0.004nHNO_3$	$0.004nHCl$	Check
1............	2	1	2.5	1	1
2............	3	2	3	2	
3............	3	3	3	2	
4............	3	3	3.5	3	
5............	3	3	4	3	

The addition of mineral acids seems to have no depressing effect, although the yields are so small that this would perhaps not be made evident. In the second series the weights were not determined, for the difference in growth is shown more strikingly by the appearance of the cultures than by the difference in weights. These cultures were made as follows:

TABLE XII

1– 5.........	No acid	Bare trace of growth
6–10.........	0.024gm per flask (=0.008*n*)	Good growth forming thin flocculent film
11–15.........	0.048gm per flask (=0.016*n*)	No germination
16–20.........	0.072gm per flask (0.032*n*)	No germination

All the acetic acid cultures fruited, so that even the minute colonies were blue with spores.

Potassium acetate.—A large number of cultures was made with potassium acetate. The data from the most significant series of those are given here.

TABLE XIII

Mineral nutrients per 100cc solution 1gm NH_4NO_3, 0.5gm KH_2PO_4, 0.25gm $MgSO_4$, 0.25gm KCl. Quantity and concentration of CH_3COOK per flask is given at the head of each column.

No.	I 0.245gm CH_3COOK (=0.05 GM. per l.)	II 0.49gm CH_3COOK (=0.1 GM. per l.)	III 0.98gm CH_3COOK (=0.2 GM. per l.)	IV 1.47gm CH_3COOK (=0.3 GM. per l.)
1....	31	41	40	40
2....	31	42	41	40
3....	32	42	41	41
4....	32	43	42	42
5....	32	43	42	51

TABLE XIV

The conditions of this series were the same as those for the preceding, except that no potassium chlorid was used in the mineral solution.

No.	I 0.245gm CH_3COOK	II 0.49gm CH_3COOK	III 0.98gm CH_3COOK	IV 1.47gm CH_3COOK
1....	30	26*	39	40
2....	31	40	40	41
5....	32	41	41	41
4....	32	41	41	41
5....	32	43	42	42

* Contaminated with a bacterium forming a green pigment.

TABLE XV

In this series the amount of CH_3COOK was 0.98^{gm} per flask, but the magnesium content was varied.

	I	II
Number	0.5^{gm} $MgSO_4$per 100^{cc}	1^{gm} $MgSO_4$ per 100^{cc}
1	54	5
2	55	14
3	55	17
4	55	20
5	56	37
6	56	39
7	56	41
8	57	47
9	57	55
11	58	64

The potassium acetate cultures show a remarkable uniformity of yield, which is independent of the concentration of the acetate, except in very dilute solutions and probably also in very concentrated solutions. The lowest concentration in *tables IX* and *X* is too dilute to allow the full development of the fungus under those conditions, but all the other concentrations give the same yield.

With the increase of magnesium in the cultures, the yield is increased but still remains uniform (*table XV*, *col. I*). A higher concentration of $MgSO_4$ becomes poisonous.

In the growth of all the potassium acetate cultures the medium becomes alkaline, showing that only the CH_3COO- radicle is taken up by the fungus. The excess of KOH (or $KHCO_3$) results in the precipitation of NH_4MgPO_4, thus keeping the culture neutral or only slightly alkaline for a time. When all the Mg is precipitated, the increased alkalinity prevents further growth. The addition of more Mg delays this period, and hence gives a greater yield. The yield is practically quantitative for a certain amount of Mg, and is independent of the amount of CH_3COOK.

The potassium acetate cultures brought out the striking difference in power of resistance to deleterious substances possessed by different spores from the same culture. This was also observed in other cases where deleterious concentrations of substances were used, but it was nowhere as evident as in the potassium acetate cultures. By the method of inoculation, it is likely that more than a thousand spores were sown in each flask. In the favorable media a large percentage germinated so that the surface was covered from the beginning with a film of fungus. In the cultures containing the stronger solutions of CH_3COOK, sometimes less than a hundred colonies were formed, but these were able to grow with sufficient vigor to produce a yield equal to that of the flasks in which more spores had germinated.

All the potassium acetate cultures formed spores, but those in the concentration of 0.05 GM. per liter produced them most abundantly.

DISCUSSION

A general survey of these data shows that alcohol, acetic acid, and the substances from which the acetic acid radicle CH_3COO- is easily derived are assimilated by *Penicillium glaucum*. In the case of alcohol the addition of mineral acids stimulates growth, but HNO_3 produces greater stimulation than HCl. The esters of alcohol with mineral acids are valueless as a source of carbon, and their lack of nutritive value is not due to any toxic properties. The substances which possess the greater food value among the foregoing are, in general, those which are readily oxidized. To what extent will these data enable us to correlate the mode of assimilation of these compounds with the known chemical reactions of the substances?

The first possibility that presents itself is that alcohol enters into combination with substances in the protoplasm by virtue of its dissociation into ethylidene and water

$$CH_3CH_2OH \leftrightharpoons CH_3CH{=}+HOH,$$

which, as Nef[10] has shown, takes place completely at about 650° C. At ordinary temperatures dissociation into ethylidene and water takes place to a very slight extent, probably less than 0.01 per cent. The dissociation, however, is enormously increased by a combination of alcohol with other substances, as with metals or mineral acids, and also by the action of enzymes and other catalytic agents. The alcoholates are dissociated to so great an extent at ordinary temperatures that they burn spontaneously in the air. Ethyl nitrate dissociates at about 200° C. (?) and potassium ethyl sulfate at 250° C. Ethyl sulfuric acid and ethyl sulfate dissociate at low temperatures, so that ether formation begins in a mixture of alcohol with a little sulfuric acid at

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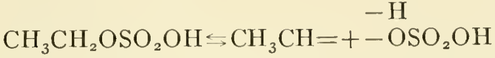
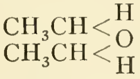
[10] Nef, J. U., On the fundamental conceptions underlying the chemistry of the element carbon. Journ. Am. Chem. Soc. 26:1549–1577. 1904.

95° C. The reaction is due, as NEF[11] has shown, to the absorption of water by the bivalent carbon of the ethylidene particles. The reaction may be represented as follows. The dissociation of ethyl sulfuric acid takes place thus:



$$CH_3CH_2OSO_2OH \leftrightarrows CH_3CH{=} + \begin{matrix}-H\\-OSO_2OH\end{matrix}$$

The sulfuric acid is regenerated and reacts with more alcohol to give ethyl sulfuric acid, or ethyl sulfate, while the ethylidene decomposes water to form ether thus:



$$\begin{matrix}CH_3CH < \begin{matrix}H\\O\end{matrix}\\CH_3CH < \begin{matrix}\\H\end{matrix}\end{matrix}$$

If, in the first steps of assimilation, alcohol enters into combination with some substance of the protoplasm to form an unknown compound, represented by $CH_2CH{=}Ppm$, then we should expect substances which increase ethylidene dissociation to affect the rapidity of assimilation. It is immaterial whether the alcohol is first elaborated into sugar, glycerin, or some other substance, before it becomes a part of the protoplasm, or whether it is directly taken up by the permanent constituents of the cell. In either case it must combine with some substance of the cell, and this combination must be regarded as the first step in assimilation.

An examination of the cultures (*tables II, III*) shows an increase in growth due to the addition of sulfuric acid, which, even in dilute solutions, would to some extent combine with alcohol and dissociate into ethylidene; but an equal increase is obtained by the addition of hydrochloric acid, and nitric acid shows an even greater increase in most cases; yet neither hydrochloric nor nitric acid combines with alcohol when mixed with it. This would indicate that stimulation by the acids is not due to any dissociating effect on the alcohol. Furthermore, if assimilation took place by direct combination of ethylidene with some substance of the cell, then bodies which dissociate very easily should be most rapidly assimilated. We find, however, that potassium ethyl sulfate and ethyl nitrate, which dissociate to a high degree, are valueless as sources of carbon when given alone, and in all probability cannot even be utilized by means of energy derived from alcohol when it is given together with the esters. This would seem to argue against the direct absorption of ethylidene by the protoplasm or cell constituents.

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[11] NEF, J. U., Dissociationsvorgänge bei den Alkyläthern der Salpetersäure, der Schwefelsäure, und der Halogenwasserstoffsäuren. Liebig's Annalen **318**:1–57. 1901.

Another possibility to be considered is the oxidation of alcohol to acetaldehyde or even to acetic acid. This view becomes the more probable on account of the ease with which alcohol is known in many instances to be oxidized by organisms. This view would perhaps explain also the increased stimulation of nitric acid over hydrochloric acid, on the basis of the oxidizing power of nitric acid. Oxidation to acetic acid, if it takes place at all, does not proceed to such an extent that the acid accumulates in the cultures.

To determine whether acetic acid was accumulated in the cultures, 10 flasks, with alcohol as an organic food, were inoculated in the usual way. After a vigorous growth took place, the culture solution was poured off, and after rinsing was replaced by a 0.3 GM. solution of alcohol. After further 6 days this liquid was tested for acetic acid, but none could be detected. DUCLAUX also was unable to observe the formation of acetic acid from alcohol by Aspergillus, but mentions the fact that alcohol was assimilated with the intermediate formation of oxalic acid. I was unable to show the presence of oxalic acid in the cultures of Penicillium, nor is it likely that this is formed. If alcohol is assimilated by way of acetic acid the oxidation takes place entirely within the cell.

Another fact gained from the ethyl acetate cultures (*table X*) speaks against the oxidation of alcohol to acetic acid. From the general principles resulting from the work on selective power or organisms by PASTEUR, DUCLAUX, and PFEFFER, we know that when two foods of different nutrient value are given to a plant, the one most readily assimilated is used, often to the exclusion of the other. Although the experiments with ethyl acetate have not been carried as far as might be desirable, there is some evidence that acid accumulates in the cultures, consequently that the alcohol radicle is more readily absorbed than the acid radicles. This, it would seem, would not take place if it was first necessary to oxidize the alcohol to acetic acid. If any oxidation takes place it is probable that this stops with acetaldehyde, from which the formation of sugars can proceed. It would then be probable that the acetic acid in all cases was reduced to the aldehyde. This would account for the greater ease of assimilation of alcohol, since the oxidation of alcohol takes place more readily than the reduction of acetic acid.

The cultures of acetic acid and potassium acetate require no discussion, since it is evident, especially from the acetate cultures, that it is the acetate ion that is assimilated. This ion was assimilated, so far as could be determined, with much more difficulty than alcohol, which would be in accord with the belief that the acid must be reduced to aldehyde.

INCIDENTAL OBSERVATIONS

As in any long series of cultures, some facts were observed in this study, which, while not directly concerned with the work, may be of sufficient interest to be worth noting.

Duclaux is responsible for the belief, current in textbooks, that some substances which permit vigorous growth of mycelia are not suitable for the germination of spores. Alcohol is one of the substances mentioned by him. Contrary to this statement, it was found that alcohol was not only favorable for growth, but also permitted abundant germination of spores. From the preliminary cultures it seems probable that all concentrations which will permit growth will also permit germination.

In many other instances substances failed to allow mycelia to develop and yet were not detrimental to germination, e. g., $C_2H_5KSO_4$. This whole subject deserves further investigation, with careful study of the effect of different concentrations on growth and germination.

An interesting observation was made on the alcohol cultures, namely, that none of them produced spores during the growth of the cultures. It is difficult at present to offer an explanation of this fact. The fungus grew more vigorously in the alcohol cultures than in any others, and as far as could be observed all the external conditions necessary for the production of spores were present. The mycelia formed dense white mats on the culture liquid, and innumerable hyphae grew into the air—a condition which usually leads to the production of spores—yet none of the aerial hyphae bore spores. It would seem as if this strain of Penicillium was unable to manufacture all of the compounds necessary for spore formation from alcohol alone;

yet such an explanation is improbable when we remember that other compounds easily derived from alcohol, e. g., acetic acid, ethyl acetate, and potassium acetate, furnish material for the development of the fungus and the production of spores. It is also possible that the suppression of spores is due to some deleterious action of the alcohol in the culture fluid, although it is difficult to see how a substance can at one and the same time act as a food of high nutrient value and as a poison. At first sight the cultures containing both ethyl acetate and alcohol would seem to bear out the view that alcohol was deleterious to spore-formation, for cultures with ethyl acetate alone fruited, while those containing alcohol also failed to fruit. In such cultures, however, it is probable that the more nutritious alcohol was absorbed first, and largely to the exclusion of the acetate. Further work is necessary to give a complete explanation of this unusual phenomenon. It may be found that all strains of Penicillium do not act alike in this respect.

Another fact, brought out in the course of this investigation, was the great individual difference of resistance of spores to deleterious agents. In the lower concentrations of all substances favorable for growth, practically all spores germinated, forming a dense matlike growth over the surface of the culture fluid. When, with increase of concentration, the substance becomes deleterious, germination and growth are not stopped abruptly, but the number of colonies becomes fewer and fewer until the final concentration is reached, where germination of even the most resistant spores is inhibited. In most cases, where only a few colonies were formed, these grew with unusual vigor, so that the total weight of the culture was often as great as that of the cultures of lower concentrations.

This was especially well shown in the series with potassium acetate, *tables XIII–XV*. Here comparatively few spores germinated in the higher concentrations, forming isolated floating colonies. Yet the yield from these was equal to the yield from other flasks which were uniformly covered. This seems to indicate that the mycelium from the more resistant spores continues itself to be more vigorous throughout life, although it is not impossible that the belief of DUCLAUX holds true here; that is, while the concentration in any given case may be injurious to germination, it does not interfere with later development.

The spores which are able to germinate then have the whole of the nutrient solution at their disposal and make more vigorous growth.

In conclusion I wish to express my thanks to Professor CHARLES R. BARNES, under whom this work was carried on; and to Professor J. U. NEF of the Department of Chemistry for many helpful suggestions relating to the chemical aspects of the work.

THE UNIVERSITY OF CHICAGO

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APOGAMY IN NEPHRODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 109
(WITH PLATES IX AND X, AND THREE TEXT FIGURES)

SHIGÉO YAMANOUCHI

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Introduction

The term apogamy was proposed by DE BARY in 1878, following FARLOW'S discovery (**21, 22**) that in *Pteris cretica*, under artificial culture, the sporophyte is developed from the gametophyte with the suppression of the sexual act. Since this discovery, the apogamous development of a sporophyte as a vegetative outgrowth from the gametophyte in pteridophytes, together with the phenomena of parthenogenesis, where the sporophyte is developed from an unfertilized egg, has been described in many forms.

FARLOW (**22**), in contrasting the apogamous embryo with the normal one, notes the following four points: (1) the apogamous embryo is intimately connected with the prothallium in such a way that one cannot decide where the one begins and the other ends; (2) there is formed no foot or equivalent organ; (3) the vascular bundle of the sporophyte is in direct connection with vessels which lie wholly in the prothallium; (4) the order of evolution is different, a leaf arising first and becoming tolerably well developed before the root and afterward the stem make their appearance.

FARLOW'S investigation was followed by an extensive study of DE BARY (**1**) on a number of forms in Polypodiaceae, in which he described a similar sporophytic growth in *Aspidium Filix-mas cristatum* and *A. falcatum*. He records various conditions of the development of sexual organs in apogamous prothallia: in *Aspidium*

JUN 13 1921

Filix-mas cristatum archegonia are apparently absent, in *Pteris cretica* they never fully developed; although all prothallia bear more or less numerous antheridia.

SADEBECK in the following year reported apogamy in *Todea africana* (in SCHENK'S *Handbuch der Botanik* **1**:233. 1879). And later apogamy was found in *Osmunda regalis* and *Ceratopteris* (LEITGEB **38**); *Todea rivularis, T. pellucida* (STANGE **63**); *Doodya caudata* (STANGE **63**, HEIM **29**); *Trichomanes alatum* (BOWER **6**); *Selaginella rupestris* (LYON **43**); *Trichomanes Krausii, Pellaea flavens, P. nivea, P. tenera, Notochlaena Eckloniana, N. sinuata, N. Marantae, Gymnogramme farinifera* (WORONIN **78, 79**); and in some others.

LANG'S study (**36**) of the apogamous development of the sporophytes on prothallia of several forms of Polypodiaceae is the most detailed contribution on apogamy in pteridophytes. The paper presents a discussion of the phenomenon in relation to alternation of generations, and adds detailed descriptions of the results of cultivating prothallia grown from spores, for a period of two years and a half in the following fourteen forms: *Aspidium aculeatum* Sw. var. *multifidum* Woll, *A. angulare* Willd. var. *foliosum multifidum,* var. *acutifolium multifidum* (no apogamy seen), *A. frondosum* Lowe; *Athyrium nipponicum* Mett., var. *cristatum, A. Filix-foemina* Bernh. var. *percristatum* Cousens, var. *cruciato-cristatum,* var. *coronatum* Lowe; *Nephrodium dilatatum* Desv. var. *cristatum gracile, N. Oreopteris* var. *coronans* Barnes; *Polypodium vulgare* L. var. *grandiceps* Fox; *Scolopendrium vulgare* Sm. var. *ramulosissimum* Woll, var. *marginale.*

According to his account, the apogamous growth resulted from artificial cultures, watered entirely from below and exposed to direct sunlight, important departures from the normal conditions surrounding fern prothallia. The asexual sporophytic outgrowth from the prothallia present some minor differences in different individuals, but, taken as a whole, they arose in the following ways: as leaves, roots, and ramenta directly on the prothallia or on a cylindrical process from the prothallium; as a continuation of the process as a leaf; as sporangia on the process from the prothallium; as tracheids in the prothallia or in the middle lobe and cylindrical process.

It seems hard to draw from these experiments any more precise conclusion than that the normal life-history is checked at a critical period (fertilization) and that the plant is forced into an expression of vegetative activity. Apogamy brings forward also the theory of the homologous origin of alternation as contrasted with the antithetic. The theory of the homologous origin of the alternation of generations, as held by PRINGSHEIM (**55, 56**) and SCOTT (**61**), is discussed by LANG in his studies on apogamy. He is inclined to the opinion that apogamy and apospory in ferns support the homologous view, since the prothallium can so readily take on sporophytic characters and the sporophyte can develop the gametophyte aposporously. He recognized, however, that all speculations on these points must be tentative until the actual nuclear conditions in apogamy and apospory have been ascertained.

The investigations mentioned above have greatly extended our knowledge concerning the phenomena of apogamy and apospory, and some of them have contributed much to elucidate the structural features involved, but cytological details of apogamy in pteridophytes remained unknown until last year, when there appeared two papers, one by FARMER and DIGBY (**24**) and the other by STRASBURGER (**68**).

FARMER and DIGBY'S paper is one of the most important of recent contributions to apogamy in ferns. Their preliminary note (**23**), published four years ago, announced the discovery of nuclear fusions in the vegetative cells previous to the apogamous sporophytic outgrowths from prothallia. Thé final paper deals with the results of their studies on apogamy and apospory in the following seven forms: *Lastrea pseudo-mas* vars. *polydactyla* Wills., *polydactyla* Dadds, *cristata apospora* Druery; *Athyrium Filix-foemina* vars. *clarissima* Jones, *clarissima* Bolton, and *unco-glomeratum* Stansfield; and *Scolopendrium vulgare* var. *crispum Drummondae*.

All the prothallia of the five forms used for their investigations, excepting the two *polydactyla* varieties, were aposporus outgrowths, either on peripheral cells of unmatured sporangia, from sori of sterile sporangia, or on apices, surface, or margins of pinnae. In two forms —var. *unco-glomeratum* and var. *cristata apospora*—the aposporous prothallia were induced artificially by pegging detached pinnae down on damp soil. The two varieties *polydactyla* of Lastrea, producing ordinary spores and prothallia, were obtained by sowing the spores.

In these apogamous prothallia antheridia are always produced in profusion and sperms are matured in every case, but the development of archegonia is different in different forms: sometimes no archegonia are produced (var. *polydactyla* Wills. and *cristata apospora*), or some are formed but do not attain maturity (var. *clarissima* Jones), and in these two cases sporophytes appeared apogamously as vegetative outgrowths; in other cases an egg is produced in the normal way, but there is no fertilization, the embryo being developed either from the unfertilized egg (var. *clarissima* Bolton and Scolopendrium) or endogenously in connection with an archegonium (*unco-glomeratum* Stansfield).

So far as the number of chromosomes is concerned, these seven cases of apospory and apogamy may be placed in three categories: (1) in the two vars. *polydactyla*, in which sporogenesis shows the ordinary reduction, a doubling of chromosomes is attained by the fusion of vegetative nuclei instead of by fertilization; (2) in four forms—three varieties of *Athyrium Filix-foemina* and a Scolopendrium—sporogenesis is omitted from the life-cycle, prothallia arise directly from abortive sporangia or pinnae, and sporophytes develop apogamously from the prothallia, the approximate sporophytic number of chromosomes being retained throughout the cycle; (3) in var. *cristata apospora*, in which apospory and apogamy regularly follow each other, the approximate number of chromosomes through the life-cycle is 60, and the authors believe that in this case the sporophyte may retain the original gametophytic number of chromosomes, the suggestion being based upon the fact that the number 60 approximates 72, the gametophyte number of the type species *Lastrea pseudo-mas*.

Throughout the investigation the authors met a variable number of chromosomes in different parts of an individual, and they express the belief that such variations are not due entirely to errors of counting, but that they correspond to fluctuations in the number actually present in the different regions. Their general conclusions are as follows: there is no necessary correlation between the periodic reduction in the number of chromosomes and the alternation of generations. Fertilization and reduction, however, are recognized as holding a very definite relation to one another, but without any *a priori* grounds for assuming any necessary connection between either of them and any other feature in the life-history; and therefore the problem of alternation must be settled by an appeal to evidence other than that derived from the facts of mitosis.

STRASBURGER (**68**) studied apogamy in Marsilia from the cytological standpoint. Previous to his work, parthenogenesis in Marsilia had been described by two observers. Almost ten years ago SHAW (**62**) found normal parthenogenesis, as it was called by the author, to be of frequent occurrence. He also isolated megaspores of *Marsilia Drummondii* from microspores before the sperms matured, and over 50 per cent. of the isolated female prothallia produced embryos, while not more than 69 per cent. of those which were mixed with male prothallia produced embryos. Four years later NATHANSOHN (**48**) induced parthenogenesis in *Marsilia vestita* and *M. macra.* He found that it was possible to stimulate the egg cell to a parthenogenetic development by exposing the germinating megaspores to a temperature of 35° C. for 24 hours, and allowing them to continue their development at a temperature of 27° C. As a result, about 7–12 per cent. of the spores gave rise to parthenogenetic embryos. Under lower temperatures the egg developed an embryo only after fertilization. No nuclear conditions were noted by SHAW or NATHANSOHN.

In parthenogenesis in seed plants, a reduction of chromosomes does not occur in the formation of the embryo sac, so that the egg nucleus contains the diploid number, which naturally obviates any necessity for the act of fertilization. Considering parthenogenesis in seed plants, there arises naturally a question as to how the egg nucleus in Marsilia, which usually establishes a new sporophyte after a normal act of fertilization, could have developed parthenogenetically into the sporophyte. A clear explanation of this question from the cytological standpoint was made by STRASBURGER in his recent paper (**68**).

STRASBURGER states that the classification into species in Marsilia cannot always be relied upon. He finds that megaspores of BRAUN'S *Marsilia Drummondii* develop embryos habitually by parthenogenesis, and that in three species—*M. vestita*, *M. aegyptica*, and *M. quadrifolia*—embryos are formed only after fertilization.

In *Marsilia Drummondii* he finds that the nuclei of the prothallia in the megaspores contain 32 chromosomes, the diploid number, as found in the root tips and other vegetative structures of the sporophyte.

One or two ventral canal cells always persist without disorganizing, which may also prevent the entrance of sperms and check the act of fertilization.

In *Marsilia vestita*, the species worked by NATHANSOHN, STRASBURGER tried NATHANSOHN's method of inducing parthenogenetic growth, but did not obtain a single parthenogenetic embryo. In this species the sporophytic number 32 is reduced to 16 in sporogenesis, and hence under natural conditions an embryo should develop only after fertilization. He found similar normal conditions in *M. quadrifolia*, *M. elata*, and *M. hirsuta*, whose chromosome number is the same as that of *M. vestita*.

According to his detailed account of sporogenesis in *Marsilia Drummondii*, the number of megaspore mother cells is less than 16, the usual number in normal forms, and sometimes only four. In diakinesis 32 chromosomes appear, and in the metaphase there were observed two kinds of mitotic figures, one of heterotypic type with 16 bivalent chromosomes, and the other of vegetative type with 32 univalent chromosomes. In both cases a second division follows, so that two kinds of megaspore tetrads are formed, one with the $2x$ or diploid number of chromosomes, and the other with the x or haploid number. The proportion of these two kinds of spores differs in each individual form; for instance, in GOEBEL's material he found the megaspores with the diploid number only. In microsporogenesis there was observed a tendency toward forming a heterotypic figure, but no mature sperms developed; two species, *M. macra* and *M. Nardu*, behaved similarly.

Such a megaspore forms a prothallium whose nuclei have the diploid number of chromosomes, which pass to the egg nuclei, so that the sporophytic number of chromosomes is maintained throughout the life-history, as in cases of parthenogenesis known among seed plants; however, the case found in *Marsilia Drummondii* by STRASBURGER, where a tetrad division occurs not accompanied by chromosome reduction, seems to be a condition never described before, because most of the cases of parthenogenesis known in seed plants are characterized by the omission of the tetrad division as well as the accompanying reduction.

STRASBURGER calls the phenomenon in Marsilia apogamy, maintaining the principle stated in his paper on parthenogenesis in EUALCHEMILLA (**66**), that the asexual development of an embryo from the gametophyte with the diploid number of chromosomes, whether the embryo comes from an egg or a vegetative cell, should be regarded as apogamy; while the term parthenogenesis should be reserved for the asexual development of an egg with the haploid number of chromosomes and consequently capable of being fertilized. WINKLER (**76**) opposed STRASBURGER'S view concerning the application of the terms apogamy and parthenogenesis. The difference of opinion concerns not only the question of terminology, but also involves theoretical views regarding the significance of the number of chromosomes, which will be considered later.

The present investigation on apogamy in *Nephrodium molle* Desv. was undertaken in the hope of adding something to our knowledge concerning the cytological interpretation of the phenomenon of apogamy. As was stated in the preliminary note, the writer was convinced of the necessity of understanding beforehand the nuclear conditions throughout the whole normal life-history of this species. Consequently, first sporogenesis, and second spermatogenesis, oogenesis, and fertilization were studied. These results were published in two preceding papers (**81, 82**).

Material and methods

All of the apogamous prothallia used in this investigation were raised from ordinary spores, secured from the same material used for the study of sporogenesis, which was collected from the greenhouses of the Hull Botanical Laboratory, of Lincoln Park, and of Washington Park, Chicago.

Spores were sown upon sterilized soil consisting of a mixture of vegetable mold and sand, placed in the greenhouse, and kept growing with special care since October 1906. Some of the prothallia presented certain peculiarities, one being different from the rest in the same pots, but in general the differences held no relation to apogamous development. Antheridia and archegonia were produced in profusion. Nuclear conditions in the vegetative cells, as well as in spermatogenesis, oogenesis, and fertilization, were studied in this material and the results were published in the preceding papers (**81, 82**).

In a number of pots placed in saucers filled with water like the rest, watering from above was avoided and the cultures were exposed to direct sunlight after the prothallia had developed two or three cells. The excessive evaporation from the soil was regulated carefully, so as not to permit condensation on the prothallia, and allow fertilization. Thus the prothallia were kept growing for a long period in dryness and in exposure to direct sunlight, the temperature of the room being kept at 28°–32° C. No fungi or lower algae developed in the pots.

The rate of growth of these prothallia when compared with that of those placed in normal conditions was quite slow. Antheridia appeared earlier than under normal conditions and were very numerous. About five or six weeks after the prothallia of two or three cells were examined, there was observed a peculiar thickening in the cushion region of some of the prothallia which reached the cushion condition earlier than the rest. This thickening was determined afterward to be the initiation of an apogamous sporophytic outgrowth. During the next three or four weeks the growth of the sporophyte was rather rapid, and at the end of that time it had become leafy. Fixation of the prothallia was made during all stages of development.

The killing and fixing of the material, with washing, imbedding, cutting, and staining, were done by the method used in the study of spermatogenesis, oogenesis, and fertilization.

This investigation was begun in October 1906, at the suggestion of Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN, and I wish to express my sincere gratitude to these gentlemen for their kind advice and criticism. I am also under obligation to the other members of the botanical staff for many courtesies.

Description of the apogamous prothallia

VEGETATIVE MITOSIS

The prothallia which produce sporophytic outgrowths apogamously do so under the influence of artificial culture. The mitoses which occur up to the 30–50-celled stage are exactly similar to those in normal prothallia, but beyond that stage the morphological structure seems to become influenced by the artificial conditions. The growth becomes very slow, and the cells show a tendency to increase greatly in size, while under normal conditions mitosis would occur before such a size had been reached. Probably for this reason mitotic figures are less frequently met during the growth of such prothallia. As the cell increases in size, its nucleus grows large and the mitotic figure is generally larger than in normal prothallia of this species.

It was not difficult to find stages of mitosis in the vegetative cells, and their comparatively large size facilitated the accurate counting of chromosomes. The resting nucleus contains a delicate reticular structure consisting of a mixture of ragged clumps and slender threads of chromatin. Nucleoli with conspicuous peculiarities of form are always present; sometimes there are two to several isolated round nucleoli scattered irregularly within the nucleus, and sometimes part of them are arranged into a group or groups. They are likely to be mistaken for chromatin nucleoli, but after a close examination of serial stages in the development of the chromosome it is clear that they lie entirely free from the chromatin network and do not seem to contribute any material to the chromosomes by direct transformation. Cell contents beside the nucleus and cytoplasm are not so abundant as in normal cases, the cell cavity consisting largely of vacuoles.

In prophase, the spirem is developed from the chromatin reticulum (*fig. 4*) as described for normal prothallia. The metaphase (*fig. 5*) and anaphase (*figs. 6, 7*) show no peculiar deviation from the typical mitosis. The number of chromosomes is 64 (*fig. 8*) or 66. When two daughter nuclei are reconstructed, a cell plate is laid down between them which finally divides the mother cell into two cells. In this material the binucleate condition was seldom observed, so that it may be claimed that the telophase of mitosis in the vegetative cell is always followed by cell division, and that there is no migration of the nucleus of one cell to an adjacent one.

As stated before, mitosis in the vegetative cells of the normal prothallia and in those reproducing apogamously agree except as to the axis of the spindle, which does not hold any regular relation to the surface of the prothallia, the cell walls being laid down in various directions. This is quite contrary to the condition in normal prothallia, in which, at least up to the appearance of the archegonium initial, the walls are formed somewhat regularly, being more or less perpendicular or parallel to the surface of the prothallium.

SPERMATOGENESIS

Every apogamous prothallium bears antheridia in profusion, the antheridium initial being formed earlier than in normal prothallia of similar size. The formation of the primary spermatogenous cell, which takes place as in normal prothallia, is studied more readily on account of the comparatively large size. The mitoses from the primary spermatogenous cell to the formation of spermatids and sperms showed no deviation from spermatogenesis in normal prothallia. During these mitoses 64 or 66 chromosomes could always be counted. The peculiar structures which were observed within the cytoplasm in the primary spermatogenous cell of the normal prothallia were also present here, but they seemed to be undoubtedly plastids.

The sperms are actively motile and are attracted by 0.01 per cent. solution of sodium malate. From the similarity of the genetic development, morphological structure, and characteristic response to the chemotactic stimulus, it is clear that sperms formed in such prothallia can function when conditions permit.

An irregularity in the axis of mitoses was observed during the cell divisions within the antheridium: in most cases the first wall which divides the primary spermatogenous cell vertically is followed by two or three vertical walls parallel to the first, before any transverse division takes place.

OOGENESIS

While antheridia and functional sperms are formed in abundance, archegonia are rare in these prothallia. The power of forming archegonia seems to be almost suppressed; and the cushion region where archegonia generally arise is very often covered with antheridia instead of archegonia. In extremely rare cases, however, there appears an archegonium initial, from which a central cell is cut off as in normal prothallia. The central cell either remains without any further division and imbedded below a superficial cell, or develops into canal cells and an egg cell, the projecting neck cells being poorly developed. The central cell, when it remains undivided, grows to a considerable size, with a corresponding increase in the size of its nucleus, which finally assumes a form similar to that of an egg, but it always appears collapsed. When canal cells and egg are formed, they also appear collapsed. The collapsed appearance of the central, canal, and egg cells is likely induced by the artificial treatment of the prothallia rather than by fixing reagents.

Whether the egg in such a collapsed condition is still capable of fertilization, is questionable, but the writer is inclined to believe that it is incapable of functioning. No case of a sperm having entered an egg was found.

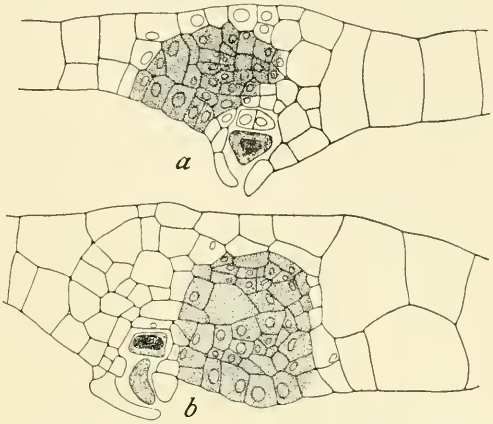

FIG. 1.—Two sections of apogamous prothallia, showing two different stages in the development of archegonia; the shaded regions represent sporophytic outgrowths; *a*, archegonium with central cell; *b*, archegonium with canal cells and egg.

Not only is the formation of archegonia extremely rare, but when formed they are very much belated. In all cases in which their formation was observed, apogamous sporophytic outgrowths were already in an advanced stage of development (*figs. 1a, b*). Hence it is reasonable to suppose that in the apogamous prothallia a tendency to develop sporophytic outgrowths becomes predominant when the power of forming archegonia becomes weak.

SPOROPHYTIC OUTGROWTHS

As described before, during the early development of prothallia, mitoses occur in the vegetative cells just as in normal prothallia, except that the mitotic figures are comparatively large. The mitoses continue in the vegetative cells and there are organized prothallia of a single cell layer in thickness. The general outline of the prothallia does not show any peculiarities which might be regarded as characteristic of apogamous prothallia as distinct from normal ones.

Sporophytic outgrowths begin very early from cells in the region where later the cushion arises, so that the development of the sporophytic outgrowth and the gradual completion of the cushion proceed side by side for a while. When the prothallium has assumed the characteristic heart shape, with a cushion near the sinus and an extensive lateral growth of prothallial tissue on either side, the sporophytic outgrowth is usually in a well-advanced stage. The main features of the formation of this outgrowth and of the gradual completion of the cushion are as follows:

Previous to the formation of the cushion, mitoses take place in rapid succession in the vicinity of the sinus, partition walls always being laid down perpendicular to the surface of the prothallium and parallel to one another, so that the cells formed are very narrow. Some of these mitoses in different stages are represented in *figs. 9–11*. Mitosis continues and cell plates are laid down parallel or oblique to the surface of the prothallium, the ultimate result being the initiation of a thick cushion region (*figs. 12, 13*).

One of the superficial cells in the cushion region begins at once to increase considerably in size, the increase being accompanied by an excessive growth of its nucleus. The nucleus in the resting condition contains a reticulum of ragged clumps and slender threads of chromatin, from which the spirem of the prophase is established (*figs. 14, 14a*). Two or more nucleoli are always present. Successive stages of the mitosis following the early prophase were examined (*figs. 15–17*), which were exactly similar to those of typical mitosis in the vegetative cells of normal prothallia. In the telophase of this mitosis, when the two groups of daughter chromosomes have reached the poles, a little irregularity in the form of the chromosomes is observed, but the number of chromosomes, before they had become aggregated into a mass, was always 64 or 66 (*figs. 18a, b*).

Consequently, it is perfectly clear that, so far as the chromatin is concerned, no change has occurred in the nucleus of these prothallia up to the formation of the superficial cell. In the late telophase a cell plate is laid down perpendicular to the surface of the prothallium, so that there are formed two superficial daughter cells arranged side by side (*fig. 19*).

A number of mitoses follow in the same way, and thus there is established in a certain region of the superficial layer a group of parallel cells. The increase of the superficial cell is remarkable in apogamous prothallia. In ordinary prothallia, if such a growth in size ever occurs in a superficial cell of the cushion region, it is an archegonium initial; however, the archegonium initial never attains such conspicuous development, surpassing all the other superficial cells in that region. Moreover, the development of the archegonium generally occurs after the cushion region has extended over a comparatively large area and attained some thickness. On the other hand, the increase in size of the superficial cell in an apogamous prothallium commences at an early stage and is associated with the gradual formation of the cushion. The accompanying figures will illustrate the situation. In *fig. 2*, *a*, *b* is shown the cushion region, displaying different stages of archegonium development in normal prothallia. *Fig. 3* represents eight successive sections of the same

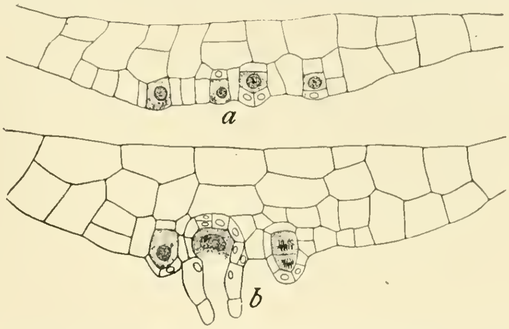

Fig. 2.—Two sections of normal prothallia, showing different stages in the development of archegonia.

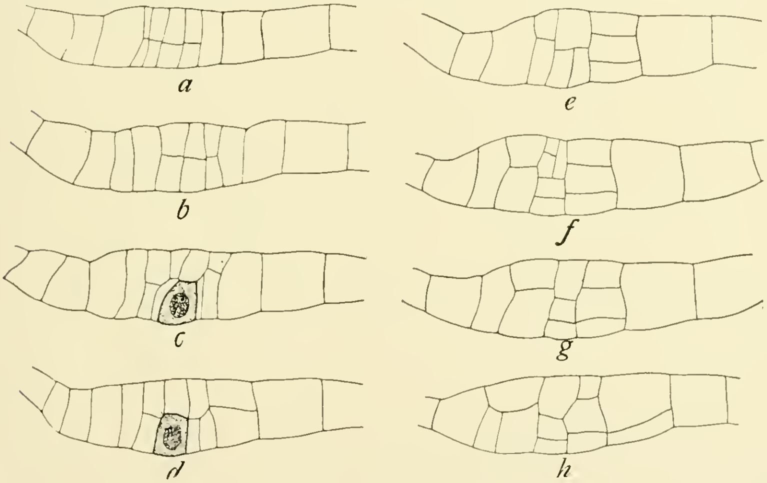

Fig. 3.—Eight successive sections of the same apogamous prothallium showing a single superficial growing to considerable size in a young cushion.

prothallium, which indicate that a single superficial cell has grown to a considerable size in a young developing cushion region. The remarkable increase in size and the characteristic contents made these cells very conspicuous. Sooner or later, from one of these superficial cells an apical cell is cut off by an oblique wall, and becomes the growing point of a leaf. Sometimes an apical cell appears very early, as shown in *figs. 20* and *21*. During these mitoses, the number of chromosomes is always 64 or 66.

While this process is in progress in the superficial region, cells beneath divide in various directions. Mitoses, of course, occur in various parts during the growth of the prothallia, but they occur more rapidly in the interior region which borders the group of superficial cells whose formation was described above.

Some of the characteristic features that distinguish these interior cells from the other prothallial cells are as follows: they are considerably smaller, probably due to their rapid successive divisions; the nucleus, in spite of the smallness of the cell, is large; the cytoplasmic contents are abundant and the plastids are small and few at this stage.

The mitoses in connection with the formation of this group of interior cells were studied. The general aspect of the mitotic figures in successive stages and the behavior of the chromosomes in these mitoses were similar to those of typical mitosis in the vegetative cells of normal prothallia. It was interesting to notice, however, that the narrowness of the cell cavity and the largeness of the nucleus showed a remarkable resemblance to the condition observed during the development of normal embryos later than the 32-celled stage (*figs. 22–29*). In telophase the number of chromosomes was always 64 or 66 (*fig. 27*).

The mitoses in the groups of superficial and interior cells continue as described above, and there is formed a structure which is in direct connection with the prothallial cells, the structure that later becomes an independent sporophyte. *Figs. 28* and *28a* represent the structure of the sporophytic outgrowth at a certain stage in the course of its development. One apical cell which is already differentiated in the structure is not shown in this section.

Thus the structure of the sporophytic outgrowth is brought about apparently by the cooperation of the mitoses of two different regions, one of which is a single superficial cell that has attained a conspicuous size, and the other the vegetative cells immediately beneath. If we trace further back the origin of the cells of these regions, they are found to be descendants from the vegetative cell or cells whose sister cells have organized the cushion region. It was impossible to detect a distinct period in which any change had occurred in the chromatin condition up to this development of the sporophytic outgrowth; practically the nucleus of the gametophyte has become directly the nucleus of the sporophytic outgrowth, without any nuclear fusion.

The sporophytic structure develops with repeated mitoses of the same sort as was described before: a leaf and a stem apex are developed from two apical cells which have appeared one after the other; a root initial is organized endogenously (*fig. 29*); scalariform vessels appear in the tissue connecting the leaf and stem origins with the root initial; and finally there is developed an independent leafy sporophyte.

From the foregoing it is clear that there is established a sporophyte with the haploid or x number of chromosomes in *Nephrodium molle*. This is the first instance yet known in plants, in which a sporophyte with the haploid number of chromosomes has been described.

Whether the sporophyte thus formed may produce spores has not yet been determined.

Discussion of cytological phenomena

APOGAMY

Since the first discovery of apogamy in *Pteris cretica*, instances of apogamy in pteridophytes have steadily increased until the phenomenon is now known in about fifty forms. No cytological studies were recorded until the appearance of the papers of STRASBURGER and of FARMER and DIGBY, already cited, but there had appeared several cytological studies of the apogamous development of the embryo in spermatophytes.

Apogamy (parthenogenesis and vegetative apogamy) is now known in spermatophytes for *Allium odorum* (TRETJAKOW 69, HEGELMAIER 28), Balanophora (TREUB 70, LOTSY 39), *Antennaria alpina*

(JUEL **30, 31**), several species of Alchemilla (MURBECK **44, 45, 46**; STRASBURGER **66**), *Thalictrum purpurascens* (OVERTON **53, 54**), *Gnetum Ula* (LOTSY **40**), several forms of Taraxacum (RAUNKIAER **57**; MURBECK **47**; JUEL **32, 33**), a number of species of Hieracium (OSTENFELD **50, 51**; MURBECK **47**; ROSENBERG **59**), and *Wikstroemia indica* (WINKLER **75, 76**). Among these contributions those of JUEL, MURBECK, OVERTON, STRASBURGER, WINKLER, and ROSENBERG present some very interesting cytological data.

JUEL (**30, 31**) made a comparative study of the parthenogenetic Antennaria and the normally fertilized *A. dioica*. In the latter a tetrad is formed from a megaspore mother cell, with the usual reduction of chromosomes, and the embryo sac is developed from one of the megaspores. There is a typical synapsis preceding a heterotypic mitosis, and the embryo sac is normal. The reduced number of chromosomes is 12–14 in the pollen mother cell and 20–24 in the integument. In the parthenogenetic *Antennaria alpina*, not only is the tetrad suppressed, but there is no trace of heterotypic and homotypic mitosis in the embryo sac. The number of chromosomes is 40–50 in the embryo sac and 45–50 in the integument. There is thus no reduction of the chromosomes during the formation of the embryo sac, and the egg retains the sporophytic number.

MURBECK (**44, 45, 46**) studied eight species of Alchemilla, chief attention being paid to the parthenogenetic *A. alpina*. In the parthenogenetic species of EUALCHEMILLA, he found that the embryo sac always developed from one megaspore of the tetrad produced from the megaspore mother cell through two successive mitoses, in which there seemed to be no evidence of a reduction of chromosomes. The number of chromosomes in these divisions is approximately 32–48, and this number is retained in the egg nucleus and the other nuclei of the embryo sac. He reports that the embryo of *A. sericata* is produced from a synergid. In the normal species *A. arvensis*, belonging to the section APHANES, he finds in the pollen mother cell 16 chromosomes—the reduced number. Two years later, MURBECK (**47**) in a short paper announced that embryos in *Taraxacum vulgare* Raunk. and *speciosum* Raunk. and *Hieracium grandidens*, *serratifrons*, and subsp. *crispatum* develop from flowers whose stamens have been removed, but he did not make any cytological studies. JUEL (**32, 33**) discovered a peculiar development of the embryo sac in the parthenogenetic *Taraxacum officinale.* The species produced pollen with a normal reduction of chromosomes, and 13 bivalent chromosomes were present in the heterotypic mitosis; but the megaspore mother cell undergoes a single mitosis and there are formed two daughter cells, the lower one of which develops into the embryo sac. This mitosis passes through the synapsis and leptonema stages as usual, but the heterotypic figure is not organized; it is a typical vegetative one with the univalent 24–26 chromosomes. The nuclear divisions in the embryo sac were not followed by JUEL, but, accepting the results of MURBECK, he believed that the egg nucleus retained 26 chromosomes—the sporophyte number.

OVERTON (**53, 54**) found a normal reduction in the pollen mother cells of *Thalictrum purpurascens* and showed that the number of chromosomes is 24 for the sporophyte and 12 for the gametophyte. The development of the embryo sac is of two types. In some cases a tetrad is formed from a megaspore mother cell, with all the phenomena of a reduction division; the lowest cell develops into the embryo sac. But many embryo sacs are formed in a different way. The first mitotic figure in the megaspore mother cell is not heterotypic, and shows 24 univalent chromosomes; and the same number is counted in the second division in the parthenogenetic embryo. He concludes that the sporophytic number (24) remains unchanged in the embryo sac in this case, and that the egg nucleus with the sporophytic number develops into the embryo parthenogenetically.

STRASBURGER (**66**) made an extensive study of numerous species of Alchemilla § EUALCHEMILLA, the group which furnished the material for MURBECK'S important discoveries. Most of the forms in EUALCHEMILLA develop normal pollen and a reduction division was found both here and in *Alchemilla arvensis* of § APHANES. In the heterotypic mitosis in the pollen mother cell, STRASBUGRER found 32 bivalent chromosomes, which MURBECK counted as 16. In the embryo sac development in apogamous species the two characteristic divisions of sporogenesis are cut out and no tetrad is formed. The nucleus of the megaspore mother cell emerges from synapsis with the sporophytic number of univalent chromosomes, and the ensuing division is typically vegetative and not heterotypic. The nuclei of the embryo sac thus contain the sporophytic number and parthenogenetic development of the egg takes place. STRASBURGER regards the parthenogenetic tendency of EUALCHEMILLA as associated with excessive mutability, which has weakened sexuality so that the process of fertilization is being displaced by apogamy.

WINKLER (**75, 76**) reports an interesting case of apogamy in *Wikstroemia indica.* He observed that seeds are produced apogamously, in spite of the fact that some pollen matures. The apogamous development of the embryo was proved by castration experiments. He describes a peculiar phenomenon in the cells of the tapetal layer, which usually contain two to six nuclei. These nuclei fuse into a huge nucleus, whose mitotic figure often shows over 100 chromosomes; but usually he counted 52 chromosomes. Although most of the pollen does not reach maturity, a tetrad division with reduction is present in sporogenesis, and in the heterotypic figure there are 26 bivalent chromosomes. The micropyle of the ovule is closed by the elongation of the inner wall of the ovary during the formation of embryo sac, which undoubtedly may have some relation to the apogamous development of the embryo. A megaspore mother cell in this form becomes directly an embryo sac, with the entire suppression of tetrad division. His material was not sufficient for a cytological study of the mitoses, and consequently he was unable to determine the entire absence of a reduction division in the embryo sac, but it seemed very likely that the egg which develops parthenogenetically may retain the sporophytic number of chromosomes.

ROSENBERG (**59**) presents the result of cytological studies on six species of Hieracium. He took up the species of Hieracium in which apogamy was proved by the experimental studies of OSTENFELD (**52**), and traced out their nuclear details. In *Hieracium excellens* the nucleus of the pollen mother cell, after it has come out from synapsis, presents a heterotypic figure with 17 bivalent chromosomes, but often with an irregularity in the number of the bivalent and univalent ones. When daughter halves of the bivalent chromosomes separate and become grouped to form the daughter nuclei, the univalent ones are left behind in the cytoplasm, as he had already observed in Drosera (**58**). In *H. flagellare* a normal heterotypic mitosis takes place in the pollen mother cell, the reduced number of chromosomes being 21. In these two species, the embryo sac develops after a tetrad division, as in normal cases; in rare cases the tetrad division with reduction is entirely cut out. The egg retaining the sporophytic number of chromosomes then develops the embryo parthenogenetically. But in the majority of cases he reports that while the normal development of the embryo sac is proceeding, an embryo sac from a cell quite near the tetrad (in *H. aurantiacum*), or in the integument, or in the chalazal region commences to develop. The normal embryo sac is then destroyed sooner or later by the encroaching embryo sac of vegetative origin. This development of an embryo sac from the nucellus is a new case, entirely different from those known in Funkia, Coelebogyne, Citrus, Opuntia, and *Alchemilla pastoralis*, for in the latter cases the embryo is produced directly from the nucellus, instead of through an intercalation of embryo sac formation, and hence the embryo is a vegetative bud from the sporophyte and entirely independent of gametophytic activities.

The papers of STRASBURGER and of FARMER and DIBGY, which are the latest contributions to the cytology of apogamy among pteridophytes, were briefly reviewed in the first part of this paper.

Summarizing the cytological facts in connection with apogamy among spermatophytes, as interpreted by different investigators, it seems evident that apogamy is closely associated with the suppression of sporogenesis in the megaspore mother cell. This necessarily results in no change in the chromosome number in the nucleus of the sporophytic generation, yet a structure is developed with the morphology of the gametophytic generation. Thus the embryo sac will contain the usual number of nuclei grouped in the typical manner, but these nuclei have the sporophytic number of chromosomes. From the facts of apospory, it seems probable then that the development of a gametophyte may result from an interference with the normal life-history and under conditions favorable to the gametophyte, even though the nuclei retain the sporophytic number of chromosomes.

If the doubling of chromosomes is that result of fertilization necessary to start the sporophyte generation, it is no surprise that either an egg with the sporophytic number or a vegetative nucleus with the same number may develop a sporophyte. In all the foregoing cases of apogamy this seems to be the situation.

The case of vars. *polydactyla* studied by FARMER and DIGBY is an instance in which the apogamous development of the sporophyte is not preceded by apospory. The prothallia in these forms are produced after normal sporogenesis and consequently they contain the gametophytic number of chromosomes. The authors claim to have found in certain vegetative cells the fusion of two nuclei, one of which has entered the cell from an adjacent one, and that the sporophyte develops from the region of the prothallium where this fusion occurs. They regard this fusion of two vegetative nuclei as a substitute for normal fertilization. A similar instance of the fusion of two vegetative nuclei is given by BLACKMAN (**4**) for Phragmidium, who regards (**4**, **5**) the process as a reduced form of fertilization. The conjugated nuclei divide simultaneously through a long series of nuclear divisions, from the formation of aecidiospores to that of teleutospores, where the last pairs unite to form the single fusion nuclei within the teleutospores. There is much evidence that the period in the life-history characterized by the presence of the paired nuclei represents a sporophytic phase. Thus the fusion of the two nuclei in vars. *polydactyla* and the pairing condition of the two nuclei in Phragmidium may support as a working hypothesis the assumption that a nucleus with the sporophyte number of chromosomes is necessary for starting the sporophyte generation.

The condition shown in the apogamous Nephrodium is entirely different from anything yet recorded for plants. The prothallia are developed after normal sporogenesis and their nuclei retain the gametophytic number of chromosomes. The sporophyte then appears as a vegetative outgrowth from the prothallium, without any visible change in the nuclei, so that there is established a sporophyte with the gametophytic number of chromosomes. FARMER and DIGBY (**24**) have suggested that the number of chromosomes, approximately 60, which is found throughout the life-cycle in *Lastrea pseudo-mas* var. *cristata apospora* may be the gametophytic number in the type species, that is 72, and that this variety might have arisen from normal prothallia of the type species through apogamy. WILLIAMS (**74**) gives an instance of true apogamous development of the egg in Dictyota, which is as yet the only type among algae where the nuclear conditions of apogamy are known. In WILLIAMS' cultures the germination of the unfertilized egg with 16 chromosomes, the gametophyte number, presented many irregularities in the segmentation divisions and most of the young embryos died after four divisions. Dictyota, then, cannot be regarded as furnishing an instance similar to Nephrodium, since the apogamous developments are abortive and it was not determined whether the structures were sporophytic or gametophytic in nature.

As regards the application of the terms apogamy and parthenogenesis in the various cases observed, STRASBURGER'S principle (**66**) is based upon the number of chromosomes contained in the embryo asexually produced; that is, the asexual development of an embryo from the gametophyte with the diploid number of chromosomes, no matter whether it originates from an egg or a vegetative cell, he calls apogamy; and he regards an unfertilized egg with the diploid number as a vegetative cell. He would restrict the term parthenogenesis to the asexual development of an egg, with the haploid number of chromosomes, and with the capability of being fertilized. WINKLER'S (**76**) view is different. He applies the term parthenogenesis to the case of an asexual development of an egg cell, no matter whether it be haploid or diploid, and he proposes to restrict the use of the term apogamy to cases in which the sporophyte is formed as a vegetative outgrowth from the gametophyte. FARMER and DIGBY'S (**24**) terminology, though not similar, resembles WINKLER'S. The terms euapogamy and parthenogenesis are applied respectively to cases of asexual development of the sporophyte from vegetative cells and from an egg cell of a prothallium produced aposporously; and to the case where a sporophytic outgrowth is preceded by the fusion of two vegetative nuclei they apply the term pseudo-apogamy. The apogamy in Nephrodium, therefore, would be called apogamy by WINKLER, euapogamy by FARMER and DIGBY, and represents no category given by STRASBURGER.

ALTERNATION OF GENERATIONS

Since HOFMEISTER'S investigations we have known that the life-history of most plants involves a regular alternation of sexual and asexual generations. The subject has been discussed by many authors, such as CELAKOWSKY (**13, 14, 15**), SACHS (**60**), BRAUN (**10**), PRINGSHEIM (**55, 56**), VINES (**72**), DE BARY (**2**), BOWER

(7, 8, 9), VAIZEY (71), STRASBURGER (65, 67), BEARD (3), CAMPBELL (11, 12), SCOTT (61), LANG (36, 37), KLEBS (34, 35), HARTOG (27), COULTER (18), DAVIS (19, 20), WILLIAMS (73), BLACKMAN (4, 5), WOLFE (77), LOTSY (41, 42), GRÉGOIRE (25), CHAMBERLAIN (16), CHRISTMAN (17), OLTMANNS (49), HARPER (26), YAMANOUCHI (80), and many others.

Among thallophytes no generalization for the whole group is possible at present, partly because of extreme diversity, and partly on account of the meagerness of our knowledge regarding the life-cycle of the majority of the forms. Different opinions are held concerning the nature of the phenomena in various forms, and some even question the existence of an alternation of generations. However, it is now being gradually established by actual investigation, and quite recently cytological proof has been obtained from several forms, as Dictyota, Phragmidium, Nemalion, Polysiphonia, and some others.

Pteridophytes and bryophytes have been regarded as the best illustrations. Discussion in connection with the pteridophytes has not been in reference to the existence of alternation, but has centered about the question whether it is to be interpreted as of antithetic or homologous origin.

These two views represent different conceptions as to the origin of the sporophyte. Those who advocate the theory of antithetic origin regard the sporophyte of pteridophytes as a gradual elaboration from the zygote of some aquatic algal ancestor, a new phase having thus been intercalated in the life-history. This view was first clearly stated by CELAKOWSKY (13, 14). BOWER (7, 8, 9) supported it and endeavored to explain it as an adaptation to external conditions. STRASBURGER (65), restating the position in terms about identical with BOWER'S, based the theory upon nuclear details. Those who maintain the theory of homologous origin consider that the sporophyte arose as a modification of the gametophyte, and not as a new structure. PRINGSHEIM (55, 56), and more recently SCOTT (61), LANG (36, 37), COULTER (18), and others advocate the homologous theory. This theory is largely based upon the phenomena of apogamy and apospory and also to a certain extent upon experiments in regeneration.

When these two theories were proposed, cytological investigations had not yet developed, and even LANG'S admirable work did not touch any cytological particulars. Since the announcement of STRASBURGER'S view of the antithetic origin of alternation, the first to be based on cytological details, chief attention has been directed by many workers to the behavior of chromosomes during the reduction division in the normal life-cycle. As to the results of such accumulated studies, the various views are not readily grouped. However, the majority of cases confirms the view that the periodic reduction of chromosomes is necessary; in other words, the gametophyte with the x number of chromosomes is entirely distinct from the sporophyte with the $2x$ number, and the transition from one generation to the other is marked by the reduction of chromosomes in sporogenesis and the doubling of chromosomes in fertilization, in connection with which the predominant characteristics of one generation are entirely lost and the potentialities of forming the other generation are regained.

As stated above, the cytological work on apogamy and apospory has been chiefly among flowering plants; and quite recently our knowledge concerning these phenomena in ferns was extended by the contribution of FARMER and DIGBY (**24**) on Lastrea, Athyrium, and Scolopendrium, and of STRASBURGER (**68**) on Marsilia. According to these investigations, apogamy, whatever its cause may be, is always preceded either by apospory or the fusion of two vegetative nuclei, which seems to favor the view that the $2x$ number of chromosomes is necessary to establish the sporophyte. Apogamy and apospory, which have been the chief argument for the theory of homologous origin, now seem to support the theory of antithetic origin.

As a matter of fact, the nuclear condition in Nephrodium in the normal life-cycle confirms the antithetic theory; but apogamy in Nephrodium introduces a new situation. In this case apogamy is preceded neither by apospory nor fusion of vegetative nuclei, but the sporophytes are developed with the haploid number of chromosomes. If it might be questioned whether the situation in Nephrodium—in which the nucleus of the gametophyte with the x number can establish the sporophyte—may favor the idea of homologous origin, it must be remembered that we have such abnormal cases of apogamy and apospory in flowering plants, where the embryo sac (probably gametophytic) does not contain the characteristic x number of chromosomes, but always the $2x$ number.

Conclusion

According to the present interpretation of the value of chromosomes, in *Nephrodium molle* Desv. there is present an antithetic alternation of generations marked by the number of chromosomes.

Apogamy in Nephrodium presents a new situation, where the sporophyte is developed with the haploid number of chromosomes. This seems to be an abnormal case, but it must be admitted that it shows that the number of chromosomes is not the only factor which determines the characters of the sporophyte and gametophyte.

THE UNIVERSITY OF CHICAGO

NOTE.—After this paper was in type, STRASBURGER'S "Chromosomenzahlen, Plasmastrukturen, Vererbungsträger und Reduktionsteilung" (Jahrb. Wiss. Bot. **45**:479–568. *pls. 1–3.* 1908) appeared. It not only presents results of investigations on *Lilium Martagon* dealing with the chromosome situation during embryo sac formation and pollen tube development, but it also contains voluminous data concerning plasma structure, chromosomes as bearers of hereditary characters, and the phenomena of the reduction division, the discussion being based upon works of various invesitgators of both plant and animal cells.

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EXPLANATION OF PLATES IX AND X

The figures were drawn with the aid of an Abbé camera lucida, under Zeiss apochromatic obj. 1.5^{mm} N. A. 1.30, combined with compensating ocular 18; except *figs. 9, 10, 11, 12, 13, 14, 20, 21, 28a* drawn with compensating ocular 4; and *figs. 28, 26,* and text cuts drawn under combination of dry obj. 4^{mm} and ocular 4. The plates and text cuts are reduced to one-half the original size.

Figs. 1–3 are in the text.

PLATE IX

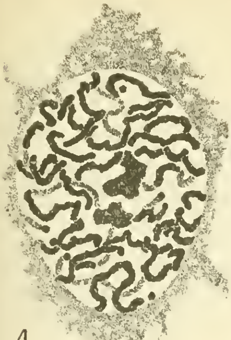
Fig. 4.—Nucleus of vegetative cell of an apogamous prothallium; spirem formed; two nucleoli present.

Fig. 5.—Late prophase; long slender chromosomes present, previous to their arrangement in an equatorial plate.

Fig. 6.—Metaphase; two daughter halves of each chromosome just separated.

Fig. 7.—Late anaphase; daughter chromosomes grouped at two poles.

Fig. 8.—Polar view of late anaphase; 64 chromosomes present in a group.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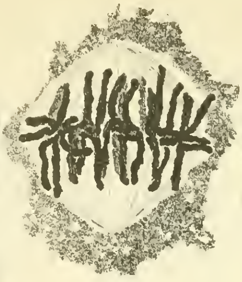
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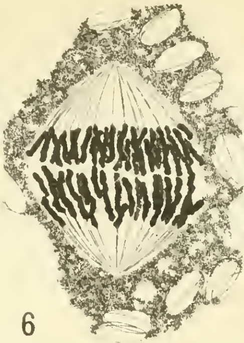
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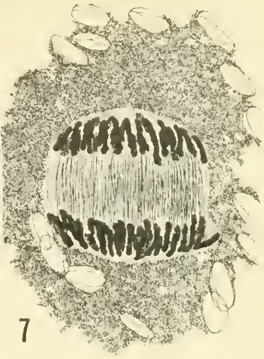
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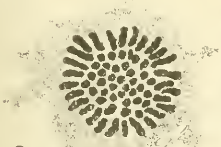
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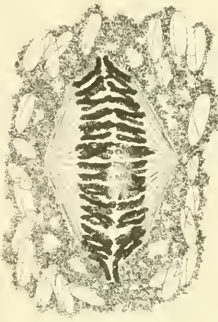
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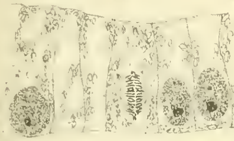
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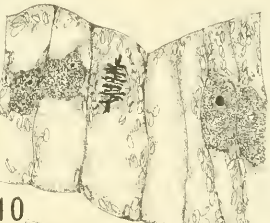
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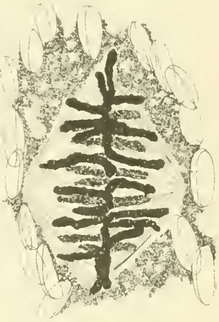
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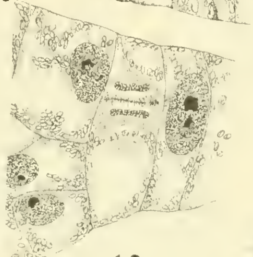
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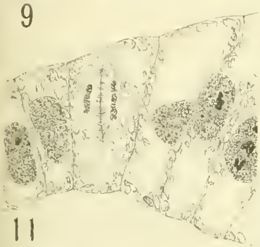
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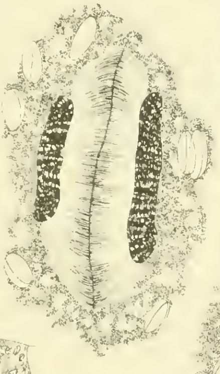
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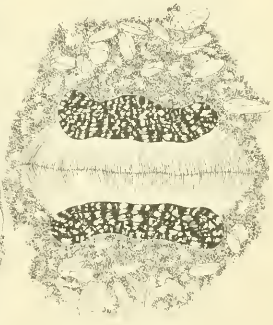
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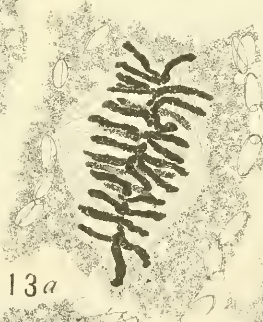
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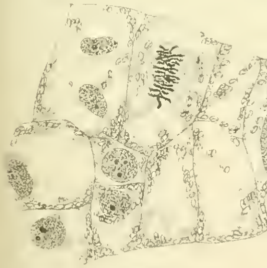
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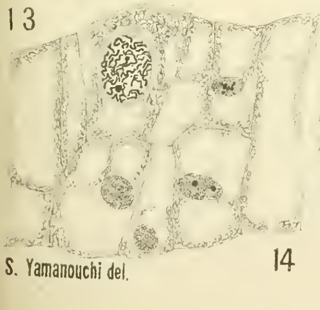
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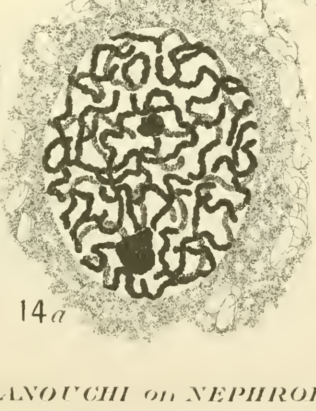
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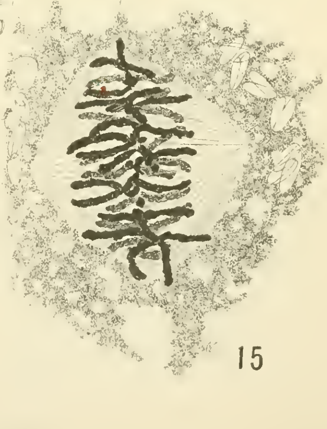
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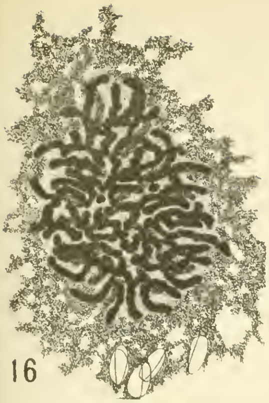
YAMANOUCHI on NEPHRODIUM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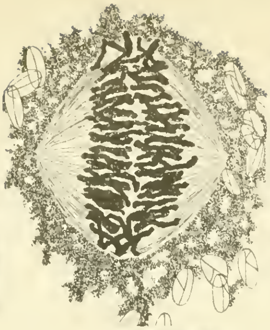
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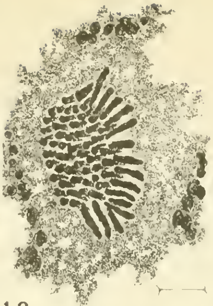
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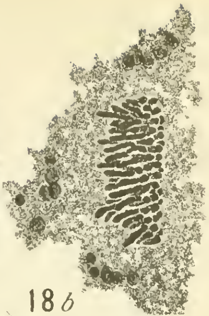
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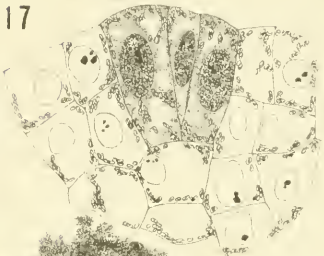
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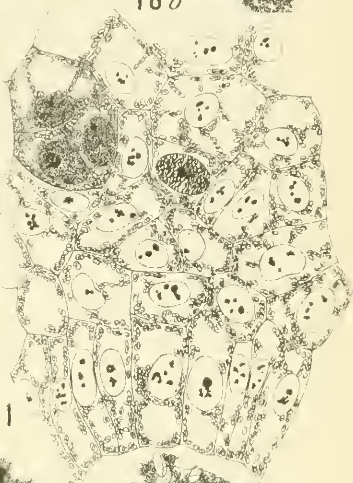
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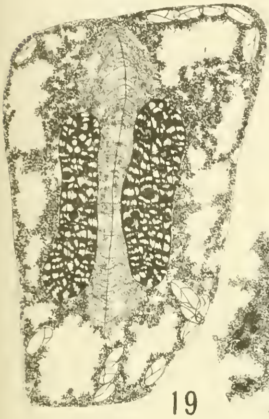
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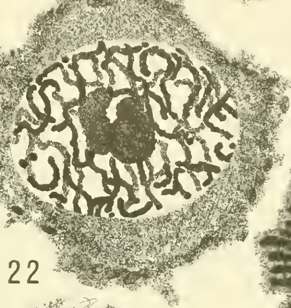
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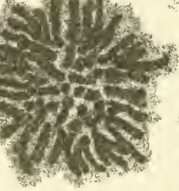
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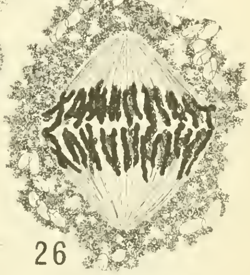
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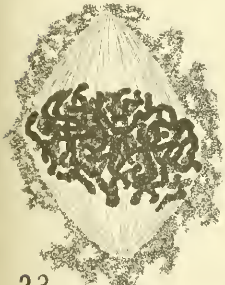
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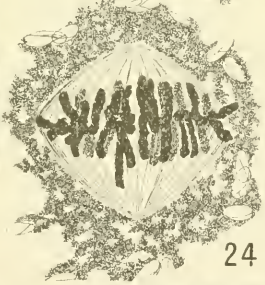
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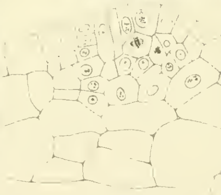
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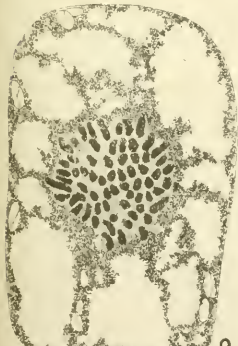
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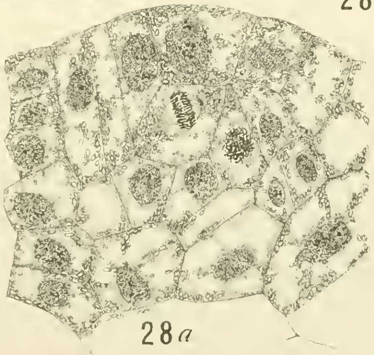
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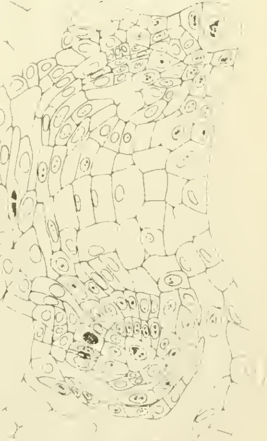
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YAMANOUCHI on NEPHRODIUM

FIG. 9.—Section through region where later a cushion arises; narrow cells parallel.

FIG. 9*a*.—Nucleus in metaphase from previous figure, under higher magnification.

FIG. 10.—Section through region forming cushion; next stage after that shown in *fig. 9*.

FIG. 10*a*.—Nucleus in late prophase from previous figure, under higher magnification.

FIG. 11.—Section through region forming cushion; later stage than that shown in *fig. 10*.

FIG. 11*a*.—Telophase of nucleus shown in previous figure, under higher magnification.

FIG. 12.—Section including cushion region; next stage after that given in *fig. 11*.

FIG. 12*a*.—Mitotic figure in telophase, shown in *fig. 12*, under higher magnification; cell plate laid down parallel to surface of prothallium.

FIG. 13.—Section of cushion region; a nucleus within superficial cell in metaphase.

FIG. 13*a*.—Nucleus in metaphase, shown in the previous figure, under higher magnification.

Mitosis in conspicuous superficial cell

FIG. 14.—One of the sections of the cushion region shown in *fig. 3* (text); one superficial cell has increased considerably in size; nucleus in prophase.

FIG. 14*a*.—Nucleus in prophase, shown in *fig. 14*, under higher magnification; several ends of the spirem seen are cut sections; two nucleoli present.

FIG. 15.—Late prophase, showing long slender chromosomes before arrangement in an equatorial plate.

PLATE X

FIG. 16.—Polar view of the late prophase shown in preceding figure.

FIG. 17.—Metaphase; two daughter halves of each chromosome just separated.

FIGS. 18*a*, 18*b*.—Two groups of daughter chromosomes in late metaphase of a nucleus cut obliquely into two sections; 66 chromosomes present.

FIG. 19.—Telophase; cell plate, vertical to surface of prothallium, laid down to cut superficial cell into two daughter cells.

FIG. 20.—Portion of section cut through cushion region; three superficial cells drawn showing contents, middle one of which is cut obliquely as an apical cell.

FIG. 21.—Surface view of a stage similar to that shown in preceding figure; the three superficial cells in question represented by drawing all the contents.

Mitosis in interior cells directly beneath superficial cells

FIG. 22.—Nucleus containing a spirem; visible ends of spirem are cut sections.

FIG. 23.—Prophase; chromosomes irregularly crowded in a mass.

FIG. 24.—Late prophase, showing chromosomes before their arrangement in an equatorial plate.

FIG. 25.—Polar view of metaphase, where L-shaped chromosomes are arranged in an equatorial plate; vertical arms of L's are visible as dots and lateral arms as radiating lines.

FIG. 26.—Late metaphase; two daughter halves of each chromosome have just separated.

FIG. 27.—Early telophase, showing polar view of a group of daughter chromosomes; 66 chromosomes present.

FIG. 28.—Section of cushion region where a sporophytic structure has been worked out.

FIG. 28*a*.—Approximate portion that belongs to the sporophytic structure shown in the preceding figure, under higher magnification; difference of size of cells in prothallial region and sporophytic structure shown.

FIG. 29.—Section through sporophyte apogamously produced, in a considerably later stage; one of the two apical cells seen is a leaf initial, the other a root initial; apical cell of stem not drawn in this section; no structure standing for a foot is present.

John M. Coulter

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RELATION OF MEGASPORES TO EMBRYO SACS IN ANGIOSPERMS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 110

JOHN M. COULTER

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RELATION OF MEGASPORES TO EMBRYO SACS IN ANGIOSPERMS

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JOHN M. COULTER

The recorded cases of unusual conditions in the angiosperm embryo sac are multiplying. Especially is this true in reference to the occurrence of more than eight nuclei in the ante-fertilization development of the gametophyte. There is a tendency to regard the latter cases as representing a more primitive condition of the gametophyte, and to exclude from consideration a general view of the genesis of the nuclei. It seems to be a fitting time to call attention to certain facts that may interpret this situation, may connect it with other "irregularities," may check the tendency to ill-considered generalization, and may indicate certain things that must be included in any investigation of the embryo sac.

In the genesis of the ordinary angiosperm embryo sac from the megaspore mother cell five successive nuclear divisions are involved, the first two being the reduction divisions (usually resulting in four megaspores with walls), and the other three resulting in the production of eight nuclei from the nucleus of one of the megaspores. The functions of these eight nuclei of the female gametophyte do not enter into the present discussion. Attention is called to the following facts: this genesis begins with the division of the mother cell; the essential part of the process is found in the first two divisions, which cannot be omitted, so far as we know, when fertilization is to occur; the result of these first two divisions is the organization of megaspore nuclei, definitely recognized by their cytological history and structure.

The most familiar illustration of departure from this ordinary sequence is the case of Lilium and its allies. In current description the mother cell of Lilium is said to "function directly as a megaspore," and the resulting eight-nucleate sac seems to be quite normal. The inference is that the formation of megaspores has been omitted, and that the nucleus of the mother cell holds the same relation to the eight nuclei of the embryo sac that the megaspore nucleus does in ordinary cases. Analysis of the situation, however, shows that this is not true, for megaspores, at least their nuclei, cannot be omitted. The first two divisions from the mother cell are reduction divisions, and therefore the first four nuclei of the embryo sac of Lilium are megaspore nuclei, to be recognized as such by their cytological history and structure. This means that the nuclei of four megaspores have entered into the structure of the female gametophyte of Lilium; and that to complete its ante-fertilization development each of these nuclei divides once. Therefore, in the case of Lilium, there is only one division after the reduction divisions, instead of the usual three; and the total number of divisions is reduced from the customary five to three. In this case the history of an embryo sac containing the usual structures shows a very unusual reduction in the number of successive divisions, and this reduction seems to have been attained by many more monocotyledons than dicotyledons.

The case of Cypripedium[1] is an illustration of a similar reduction; but with the old methods of interpretation it would stand as remarkably different from Lilium and most angiosperms, for the completed embryo sac, ready for fertilization, contains only four nuclei. But in this case the mother cell divides, and one of the daughter cells forms the embryo sac. The first division of the nucleus of this daughter cell is the second reduction division, resulting in two megaspore nuclei. It is these two megaspore nuclei that are involved in the development of the female gametophyte, each dividing once as in the case of Lilium. Therefore, in Cypripedium also the usual five successive divisions are reduced to three; the only difference being that in Lilium four megaspore nuclei are involved, while in Cypripedium only two are used. In her paper Miss PACE calls attention to this comparison, and also to the fact that the elimination of one more division would result in an oogenesis similar to that of animals.

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[1] PACE, LULU, Fertilization in Cypripedium. BOT. GAZETTE 44:353–374. *pls. 24–27.* 1907.

In the older papers many records of "three megaspores," "two megaspores," and "no megaspores" appear, which either must be due to incomplete investigation or represent such an elimination of divisions as is shown by Lilium and Cypripedium.

What has been regarded as the most striking exception to the usual structure of the angiosperm embryo sac is the case of *Peperomia pellucida*, described by CAMPBELL[2] and JOHNSON.[3] In this sac sixteen nuclei appear before fertilization; that is, there appears to be one more division than usual. Lately JOHNSON[4] has described a similar condition in the Jamaican *Peperomia hispidula*, in which there are also eight-nucleate sacs.

Fortunately, in the case of Peperomia we know that the mother cell does not divide to form walled megaspores, so that the probable interpretation of the situation is evident. Four megaspore nuclei are formed, all of which are probably involved in forming the female gametophyte. By two successive divisions each of these gives rise to four nuclei, and the result is sixteen nuclei. It follows that the ordinary number of successive divisions has been reduced from five to four; and that if Peperomia had suffered no reduction, there would have been thirty-two nuclei in the embryo sac. In number of divisions, therefore, Peperomia lies between the ordinary angiosperms on the one side, and Lilium and Cypripedium on the other. This condition should be regarded as advanced in the direction of the elimination of divisions, rather than primitive. If an embryo sac derived from a single megaspore should contain sixteen nuclei, it could be regarded as relatively primitive; but the sac of Peperomia could contain thirty-two nuclei and only be normal. *Peperomia hispidula* is interesting in developing eight-nucleate sacs in addition to the sixteen-nucleate ones, which perhaps puts it upon a level of reduction with Lilium. It may have some bearing on the situation to note also that JOHNSON in his account of this species described the first four nuclei of the sac as "arranged in a perfect tetrad," which would be quite natural for megaspores. In fact, it may not be out of place to state that JOHNSON has expressed the opinion privately that these four nuclei are those of megaspores.

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[2] CAMPBELL, DOUGLAS H., Die Entwicklung des Embryosackes von *Peperomia pellucida* Kunth. Ber. Deutsch. Bot. Gesells. **17**:452–456. *pl. 31.* 1899.

[3] JOHNSON, D. S., On the endosperm and embryo of *Peperomia pellucida.* BOT. GAZETTE **30**:1–11. *pls. 28, 29.* 1900.

[4] JOHNSON, D. S., A new type of embryo sac in Peperomia. Preliminary notice. Johns Hopkins Univ. Circ. **1907**:19–21. *pls. 5, 6.*

Recently CAMPBELL[5] has examined *Pandanus odoratissimus* and *P. Artocarpus*, and found usually fourteen nuclei in the embryo sac. At the four-nucleate stage there is the usual arrangement of two micropylar and two antipodal nuclei; but the former do not divide, and the latter give rise to twelve nuclei. If this record is confirmed by tracing the history of the nuclei, there is introduced in this case an irregularity in the succession of divisions. It may be assumed, subject to subsequent observation, that four megaspore nuclei are involved in the formation of these fourteen nuclei; and the irregularity would consist in the fact that two of them do not divide; that one of the others added two successive divisions (the elimination of one division); and that the remaining one added three successive divisions (the usual number). There is thus no greater number of divisions than usual, and even a certain amount of elimination. It is interesting to note that if the two micropylar nuclei do not divide and are really megaspore nuclei, the egg is removed from the mother cell by only two divisions, which is the condition of the animal egg. A careful cytological investigation of these divisions would seem to be most desirable.

The introduction of a certain amount of irregularity in the succession of divisions is suggested by the records of several species. For example, in Gunnera,[6] while eight nuclei are common, any number up to sixteen may be found; and in *Trillium grandiflorum*[7] ten sometimes occur. In neither of these species, however, do we have any information as to the behavior of the mother cell in forming megaspores.

In *Ulmus americana*[8] we know that the "mother cell functions directly as the megaspore," as in Peperomia, and in the majority of embryo sacs there are eight nuclei (the normal number of nuclei, but an elimination of two divisions); but sometimes there are "twelve or more nuclei" (meaning another division, but still one less than the usual number).

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[5] CAMPBELL, DOUGLAS H., The embryo sac of Pandanus. Preliminary note. Annals of Botany **22**:330. 1908.

[6] SCHNEGG, H., Beiträge zur Kenntniss der Gattung Gunnera. Flora **90**:161–208. *figs. 28.* 1902.

[7] ERNST, A., Chromosomereduction, Entwicklung des Embryosackes, und Befruchtung bei *Paris quadrifolia* L. und *Trillium grandiflorum* Salisb. Flora **91**: 1–46. *pls. 1–6.* 1902.

[8] SHATTUCK, CHARLES H., A morphological study of *Ulmus americana*. BOT. GAZETTE **40**:209–223. *pls. 7–9.* 1905.

The situation among the Araceae deserves attention, for great irregularities in the embryo sac have been observed.[9] In Aglaonema the nuclei are four to twelve; while in Nephthytis they are reported as ranging in number from two to twelve or thirteen. Unfortunately, in these cases we are not sure of the behavior of the mother cell; but so far as the records for Araceae go, the mother cell divides once or not at all. It is needless to draw any conclusions as to the amount of reduction involved in the four nuclei of Aglaonema or the two nuclei of Nephthytis until we know something of their history. At all events, there is a reduction of the ordinary number of divisions in these forms; and this is probably true even in the cases where twelve or thirteen nuclei appear.

The only case reported, in which there remains some evidence that an additional free nuclear division may occur, is that of the Penaeaceae, a small African group of shrubby xerophytes restricted to southwestern Cape Colony. In a preliminary note, STEPHENS[10] has announced his results with five species, representing three genera (Sarcocolla, Penaea, and Brachysiphon), and his account applies to all of them. There are sixteen free nuclei formed in the sac before fertilization, which are said to be derived from the innermost one of a row of three megaspores. In such a case three "megaspores" are as good as four, except that the outermost cell is not a megaspore. The testimony breaks down, however, at the critical stage, for the investigator is not certain as to the "row of three." Of course if it should turn out to be a "row of two," the megaspore condition would be that of Cypripedium, followed by the usual number of divisions. The behavior of the nuclei, however, suggests strongly that four megaspore nuclei are concerned, and that there is no "row" developed by the mother cell at all. It is a very interesting performance as described; for the original nucleus of the sac produces four nuclei in tetrahedral arrangement; these four separate and become parietally placed (one micropylar, one antipodal, two equatorial); each of the four produces a group of four nuclei; three nuclei of each group organize after the fashion of an egg apparatus; and the four free nuclei pass to the center of the sac and fuse to form the primary endosperm nucleus. We have here at least the assurance of simultaneous successive divisions, which we do not have in the case of all 16-nucleate sacs. If four megaspore nuclei are involved in this organization, the reduction in the number of successive divisions is just that which probably occurs in Peperomia.

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[9] CAMPBELL, DOUGLAS H., Studies on the Araceae. The embryo sac and embryo of Aglaonema and Spathicarpa. Annals of Botany **17**:665–687. *pls. 30–32.* 1903.

———Studies in the Araceae. III. Annals of Botany **19**:329–349. *pls. 14–17.* 1905.

[10] STEPHENS, E. L., A preliminary note on the embryo sac of certain Penaeaceae. Annals of Botany **22**:329, 330. 1908.

Some evident conclusions may be drawn from the above statement, and they should be of use in investigations of the angiosperm embryo sac. The nuclear divisions within the embryo sac vary in character; they may include both reduction divisions, the second one, or neither of them (the usual case). The appearance of more than eight nuclei in an embryo sac is no evidence that there has been any more than the usual number of successive divisions. As many as thirty-two nuclei may appear in an embryo sac without increasing the usual number of divisions. It is absolutely necessary to trace the nuclear succession from mother cell to completed embryo sac before any safe conclusions can be drawn as to the significance of the conditions observed within the sac. The cases of an increased number of nuclei, as in Peperomia, which have sometimes been cited as showing a primitive condition of the sac, have actually turned out to be cases of reduction from the ordinary condition. There is a tendency among many groups to eliminate the divisions that follow the two reduction divisions, but the tendency is not general among angiosperms, since among the Sympetalae it does not seem to exist; hence the forms which exhibit it may be regarded as more or less specialized in this feature.

If an angiosperm embryo sac should be found containing sixteen or more nuclei derived from a single megaspore nucleus, it may be regarded as relatively primitive; but such a sac has not yet been demonstrated.

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THE EMBRYO OF CERATOZAMIA: A PHYSIOLOGICAL STUDY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY III

(WITH SEVEN FIGURES)

HELEN A. DORETY

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THE EMBRYO OF CERATOZAMIA: A PHYSIOLOGICAL STUDY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **111**

(WITH SEVEN FIGURES)

Ceratozamia is a genus of cycads of the American tropics. Its habit and habitat and the manner in which the ovules are shed shortly after fertilization are described by CHAMBERLAIN (**1**) in his preliminary note to the study of the reproductive structures.

The embryo has only one cotyledon. This fact was observed by VAN TIEGHEM (**2**) as early as 1873 in a form which he considered a hybrid between *C. longifolia* and *C. mexicana*, but which was probably pure *C. longifolia*. In 1878 WARMING (**3**) recorded the monocotyledonous condition of the embryo of *C. mexicana*, adding that the cotyledon arises at one side of the hypocotyl axis and little by little comes to surround it.

Being engaged in an anatomical study of the seedling, the first observation I made was naturally upon this character. In every case in over one hundred ovules germinated in 1906, the embryos bore only one cotyledon (*figs. 1, 2, 3*).

When the anatomical study was completed, a preliminary note was received from MATTE (4), who had germinated some seeds provided by CHAMBERLAIN and had begun the study, not knowing that the work was being carried on in this laboratory. MATTE reports that the embryo is monocotyledonous. CHAMBERLAIN (14) in his recent report on Ceratozamia, read before the Chicago meeting of the Botanical Society of America, makes the same statement.

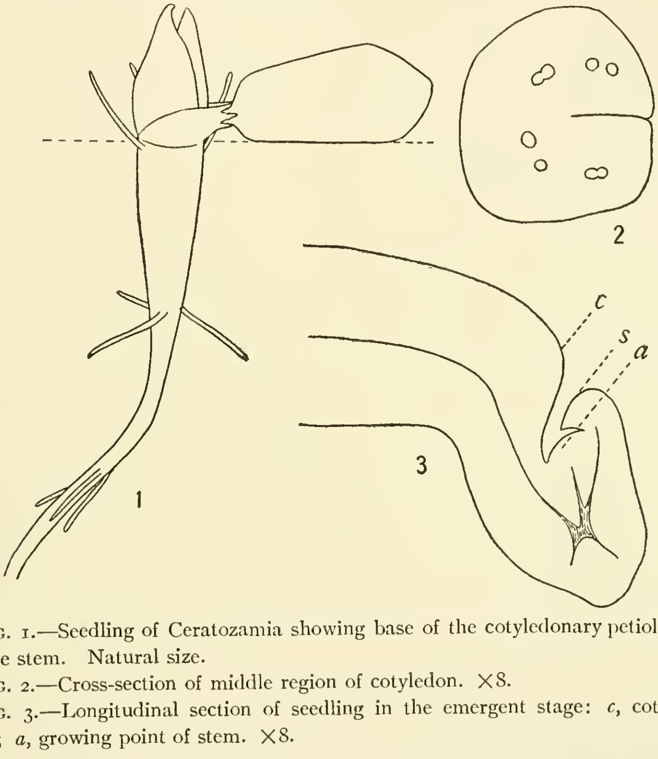

FIG. 1.—Seedling of Ceratozamia showing base of the cotyledonary petiole encircling the stem. Natural size.

FIG. 2.—Cross-section of middle region of cotyledon. ×8.

FIG. 3.—Longitudinal section of seedling in the emergent stage: *c*, cotyledon; *s*, scale; *a*, growing point of stem. ×8.

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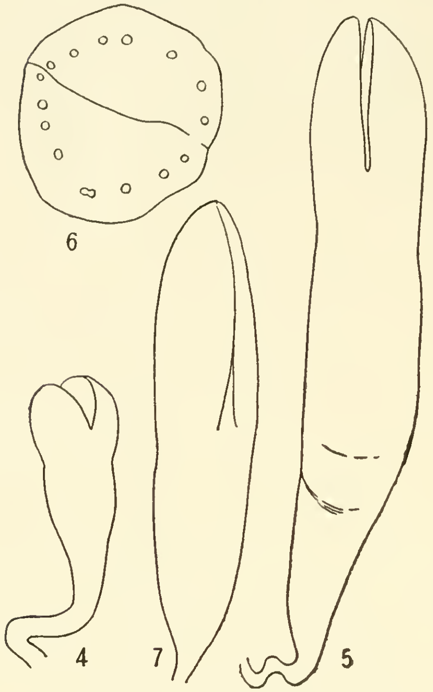
In other cycad embryos, the cotyledons are often reported as unequal in size. TREUB (5) observed and figured the inequality in *Cycas circinalis*. WORSDELL (6) described a seedling of *Cycas revoluta* in which one cotyledon was considerably shorter than the other. MATTE (7) reports a case in *Dioon edule* in which the vascular strands in the smaller cotyledon are reduced in number, only the rudiments of the missing ones occurring at the base of the cotyledon. He also mentions an inequality in the size of the cotyledons of *Encephalartos Barteri.* BOWER (8) recorded a difference in the size and anatomy of the two cotyledons of *Cycas Seemanni;* and LAND (Chicago) has noted (unpublished) a difference in the size of the cotyledons of *Zamia integrifolia.*



FIGS. 4 and 5.—Embryos developed on clinostat; removed February 3. ×8.

FIG. 6.—Cross-section of middle region of cotyledons of an embryo removed from clinostat February 26. ×8.

FIG. 7.—Embryo taken from soil in green house January 8. ×8.

Outside the cycads one finds the same tendency in seeds of similar habit. SOLMS-LAUBACH (9) figured it in an embryo of *Bennettites Gibsoniana,* a fossil relative of the cycads; and COULTER and CHAMBERLAIN (10) and LYON (11) have recorded it of Ginkgo.

In the course of my anatomical study, I noticed that the cotyledon was a lateral structure and that there was absolutely no external trace of another (*fig. 3*), but certain irregularities in the vascular system made me suspect that the second cotyledon had been suppressed. These irregularities consisted mainly in the suppression of one pole of the root, and the presence of certain rudimentary strands which joined the stem in normal fashion, but whose upper portions ended blindly in the cortex or fused with other bundles. The suspicion was strengthened by the observation that in every case the cotyledon developed on the under side as the seed lay. This latter circumstance led me to think that gravity was responsible for the monocotyledonous condition.

In the following autumn, therefore, fresh ovules were placed in broad pots, covered closely with sphagnum, and placed upon clinostats revolving at different rates of speed. In December the LIVINGSTON porous cup apparatus (12) was inserted in order to equalize the soil moisture. At the same time fresh seeds were planted in the greenhouse.

The apparently simple plan of planting the seeds end down was not adopted for two reasons. First, this would not give accurate results, because such a seed as that of Ceratozamia could not be placed and kept in an exactly vertical position, and there is no doubt that a variation from the vertical of only a few degrees is sufficient to bring an organ under the direct influence of gravity. Second, at the time of formation of the growing points, the embryo has been pushed half-way through the massive endosperm in a tortuous and winding path by the elongating suspensor, and it is probable that just at the time when it comes to rest, gravity will determine its position whether the seed be vertical or horizontal.

Rotation on the clinostat seems to have retarded development to a marked degree, but has not otherwise interfered with it. Early in February, the embryos developing on the clinostat were found to have two equal cotyledons (*figs. 4, 5, 6*), while those developing under normal conditions have only one (*fig. 7*). Further, the dicotyledonous embryos have a tetrarch stele in the root and the cotyledonary vascular system is of the usual cycad type.

It can scarcely be doubted that gravity is the main factor in bringing about the inequality reported in the cotyledons of other gymnosperms, and it may be that further experiment will throw some light upon the seeming suppression of root poles. It is possible also that it is to this cause we may attribute not only the monocotyledonous condition long known to exist in some dicotyledons, mentioned by COULTER and CHAMBERLAIN (**13**); but even the condition of certain true monocotyledons whose embryos from their very inception hold a practically unchanged one-sided relation to gravity.

The experimental work is under the direction of Professor CHARLES R. BARNES and Dr. WILLIAM CROCKER.—HELEN A. DORETY, *The University of Chicago.*

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Compliments of
John M. Coulter

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THE EMBRYO SAC AND EMBRYO OF *GNETUM GNEMON*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 112

(WITH PLATE VII)

JOHN M. COULTER

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THE EMBRYO SAC AND EMBRYO OF *GNETUM GNEMON*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 112

JOHN M. COULTER

(WITH PLATE VII)

Gnetum Gnemon has been made conspicuous among the other species of Gnetum chiefly by the investigation of LOTSY published in 1899.[1] The structures described were of such interest that it seemed desirable to supplement the somewhat incomplete account by a further examination. Accordingly material was obtained from the Philippine Islands and from British Guiana; the former from Dr. H. N. WHITFORD, and the latter from Mr. A. W. BARTLETT, director of the Botanic Garden at Georgetown. This material was first assembled by Dr. W. J. G. LAND, of this laboratory, in connection with his investigation of Ephedra; and he has kindly turned it over to me for separate study. The preparations and drawings were made by Dr. SHIGÉO YAMANOUCHI, of this laboratory, and to his technical skill the results are largely due.

The material included stages from two successive seasons, but unfortunately many intervening stages were not represented, so that no continuous account can be given. However, certain facts have been discovered that supplement and correct the previous accounts.

EMBRYO SAC

LOTSY described the embryo sac of *G. Gnemon* as showing an interesting deviation from those found by KARSTEN[2] in other species of Gnetum. Instead of containing only free nuclei at the fertilization stage, the embryo sac of *G. Gnemon* was described as containing a compact antipodal tissue, sharply distinct from the micropylar chamber with its free nuclei. As a consequence, the embryo sac of *G. Gnemon* has been used ever since as illustrating a female gametophyte intermediate in structure between the tissue-filled sacs of Ephedra and Tumboa on the one hand, and the sacs of other species of Gnetum, which contain only free nuclei. Later the same investigator in reporting parthenogenesis in *G. Ula*[3] described the embryo sac of that species as being of the *G. Gnemon* type.

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[1] LOTSY, J. P., Contributions to the life history of the genus Gnetum. Ann. Jard. Bot. Buitenzorg II. 1:46–114. *pls. 2–11.* 1899.

[2] KARSTEN, H., Bot. Zeit. 50:205–215, 221–231, 237–246. *pls. 5, 6.* 1892; Ann. Jard. Bot. Buitenzorg 11:195–218. *pls. 17–19.* 1893; Cohn's Beitr. Biol. Pflanzen 6: 337–382. *pls. 8–11.* 1893.

Our material of *G. Gnemon* does not confirm this account. At an early stage of the embryo sac, eight nuclei are observed grouped near the center (*fig. 1*), the sac being invested by the loose tissue of the nucellus. At a somewhat later stage the nucellar cells at the chalazal end of the sac are strikingly differentiated (*fig. 2*), becoming more and more compactly arranged, gradually obliterating the intercellular spaces, and taking on the appearance of glandular cells. The relation of this tissue in its early stage to the embryo sac is shown in *fig. 2a*. As vacuolation proceeds in the sac and the free nuclei become parietally placed, this "pavement tissue" becomes more compact and extends deeper into the chalaza (*figs. 3, 3a*). Still later it spreads laterally below, until it becomes fan-shaped in section (*figs. 4, 4a*), but it is always very distinct in contour and sharply marked off from the surrounding nucellar tissue. At the fertilization stage (*figs. 4, 5*) the sac contains only free nuclei, which become somewhat grouped at the antipodal end (*fig. 5*), but there is no walled tissue. Spreading below the sac, however, the mass of nucellar pavement tissue shows a definite contour, which might be merged in imagination with that of the sac and thus mistaken for a compact tissue within the antipodal end of the sac. LOTSY'S figures show the real contour of the sac, and his antipodal tissue is clearly this glandular pavement tissue developed in the chalaza. So far as the sac of *G. Gnemon* is concerned, therefore, its fertilization stage is that described for other species of Gnetum. It will be noted that after the fertilization stage is reached (*fig. 5*) the pavement tissue begins to lose its glandular character; and later it is destroyed entirely by the growing endosperm.

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[3] LOTSY, J. P., Parthenogenesis bei *Gnetum Ula* Brongn. Flora **92**:397–404. *pls. 9, 10.* 1903.

ENDOSPERM

A year later, the endosperm has destroyed all of the nucellar tissue except a very small amount at the tip (*figs. 6, 6a, 7a*), and it is clearly differentiated into a central region of smaller, more compact cells, and a more extensive peripheral region of larger and looser cells. In destroying the nucellar beak, a curious result is observed. The central region of the endosperm advances into the beak and then spreads laterally (*fig. 6*). In the meantime the peripheral region advances more slowly toward the beak, and as a consequence a ring of nucellar tissue is pinched between two growing masses of endosperm. The growth of the endosperm into the chalazal region also results in pressure toward the beak, so that the pinched nucellar tissue is under considerable pressure and becomes completely disorganized. Under this pressure some of the adjacent endosperm cells also become disorganized.

In ovules of the preceding year, at the fertilization stage of the embryo sac, a curious disorganization of some of the cells of the nucellar beak was observed (*fig. 5a*). Two transverse rings of cells, several layers beneath the epidermis, begin to disorganize; later the epidermis becomes involuted between the disorganized rings, resulting in a deep groove around the nucellus. The epidermal cells remain very vigorous in appearance, and when the endosperm develops into this region the groove disappears. The cause and the significance of this disorganization and of the temporary involution of the epidermis cannot be suggested.

EMBRYO

Lotsy has described the entrance of pollen tubes into the embryo sac, the fertilization of the free eggs, the excessive elongation of the fertilized eggs to form suspensors, and the cutting-off of the embryonal cells at the tip of the suspensor. Later stages in the development of the embryo have been described by Bower,[4] but the intermediate stages have not been observed. Fortunately our material from the Philippines contained them, and revealed an unexpected situation.

When the endosperm has become fully developed, its peripheral region contains a tangle of long, tortuous, and branching suspensors (*figs. 6, 7*), which are difficult to trace. During the formation of a suspensor by a fertilized egg, free nuclear division occurs, resulting in a few nuclei (four in *fig. 7*) distributed along the suspensor. Usually between these nuclei transverse walls are formed by the development of a cleavage plate from the wall of the suspensor. A cell at the tip of the suspensor is cut off in the same way, and contains one of the free nuclei, which becomes associated with numerous starch grains (*fig. 7*).

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[4] Bower, F. O., The germination and embryology of *Gnetum Gnemon*. Quart. Jour. Micr. Sci. **22**:278–298. *pl. 25*. 1882.

In this terminal embryonal cell free nuclear division continues (*figs. 8, 9, 10*), accompanied by cleavage walls, until a multicellular embryo is formed. In *figs. 9* and *10* it will be observed that this cleavage apparently continues until uninucleate cells are produced; and in our material this stage is reached first by a group of cells on one side of the embryo. It could not be determined whether this group holds any relation to a body region or not.

It has been supposed that in the embryogeny of Gnetum the preliminary stage of free nuclear division, common to other gymnosperms, had been eliminated; and that the first nuclear division was accompanied by wall formation, as in angiosperms. In *Gnetum Gnemon*, however, free nuclear division not only characterizes the proembryo, but also the early stages of the embryo. The case may be compared to that of Ephedra,[5] in which free nuclear division within the fertilized egg results in eight independent proembryonal cells, each of which continues free nuclear division and develops as a suspensor, which by a cleavage wall cuts off the terminal embryonal cell. In Gnetum the suspensor is formed by the fertilized egg instead of by a proembryonal cell, but the number of free nuclei formed by the egg in each case is approximately the same.

INTEGUMENTS

The mature seed of *Gnetum Gnemon* gives an opportunity to compare the integument and testa with those of other gymnosperms. *Fig. 6a* shows the seed slightly stalked within the so-called "perianth," which is fleshy. Two integuments are evident, and they develop in basipetal succession. The inner one extends above to form the elongated micropylar tube, and at the maturity of the seed completely invests the nucellus (at this time replaced by the endosperm) as a papery layer. The outer integument becomes differentiated into an outer fleshy layer (white in the figure) and an inner stony layer (black in the figure), the latter completely investing the seed, the former being chiefly developed in the region of the nucellar beak. Two sets of vascular strands are present, the outer set traversing the fleshy layer of the outer integument, the inner set traversing the inner integument.

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[5] LAND, W. J. G., Fertilization and embryogeny in *Ephedra trifurca*. BOT. GAZETTE 44:273–292. *pls. 20–22.* 1907.

In Cycadophytes, Ginkgoales, and Coniferales, a single integument becomes differentiated into a testa of three layers: outer fleshy, stony, and inner fleshy. In Gnetum the same three layers are present, but the inner fleshy one has become differentiated in ontogeny as a separate integument. In all cases, this innermost layer finally forms a papery lining of the stony layer. Among the Pinaceae the outer fleshy layer is present in the integument, but it does not develop into the extensive pulpy investment that characterizes the Cycadales, Ginkgoales, and Taxaceae, a fact which is probably associated with the close investment of the seeds by the scales.

The variation in the distribution of the vascular strands among these layers is interesting. Among the more primitive Cycadofilicales and Cordaitales, in which the nucellus is relatively free from the integument, the outer set of strands traverses the outer fleshy layer and the inner traverses the peripheral tissue of the nucellus. In other Cycadofilicales and Cordaitales, however, and in Cycadales in which the nucellus and integument are free only in the region of the nucellar beak, the inner set of vascular strands traverses the inner fleshy layer of the integument; and this is the condition in Gnetum, except that this layer has become differentiated as an inner integument. In Ginkgoales the outer set of strands (belonging to the outer fleshy layer) is suppressed; in Taxaceae the inner set (belonging to the inner fleshy layer) is suppressed; and in Pinaceae both are suppressed.

MALE GAMETOPHYTE

It was a disappointment that the development of the male gametophyte was not secured, for it is only known among Gnetales in Ephedra, in which it has been described by LAND.[6] The development of the tetrad was observed; and although the early anaphase of the first mitosis was not available for the counting of chromosomes, the late prophase of this mitosis and the anaphase of the second mitosis showed clearly that the chromosome numbers are 12 and 24.

SUMMARY

1. The "antipodal tissue" described by LOTSY as occurring in *Gnetum Gnemon* at the fertilization stage is a sharply differentiated nutritive tissue developed in the nucellus beneath the embryo sac, which at this stage contains only free nuclei, as described for other species of Gnetum.

2. Embryo formation begins with an excessive, suspensor-like elongation of the fertilized egg, accompanied by free nuclear division and cleavage walls; and the continuation of free nuclear divisions and cleavage walls in the embryonal cell until a multicellular embryo is formed.

3. The endosperm encroaches upon the tissue of the nucellar beak with some irregularity, an irregularity which reaches its extreme expression in Torreya, with its so-called "ruminated" seeds.

4. The inner integument of the ovule is the morphological equivalent of the "inner fleshy layer" of the single integument of other gymnosperms; and the occurrence of two sets of vascular strands is a relatively primitive condition, which has been departed from by Ginkgoales and Coniferales.

5. The chromosome numbers are 12 and 24.

THE UNIVERSITY OF CHICAGO

EXPLANATION OF PLATE VII

FIG. 1.—Embryo sac at an early stage, with centrally placed group of eight free nuclei; the cell above, with a large nucleus, is another embryo sac. ×500.

FIG. 2.—Somewhat later stage of embryo sac (all the nuclei not included), showing the beginning of the formation of the pavement tissue; a second embryo sac is also shown. ×500.

FIG. 2*a*.—An ovule at an early stage, showing the two integuments and the relation of the pavement tissue and embryo sac to the nucellus at the stage shown in *fig. 2*. ×40.

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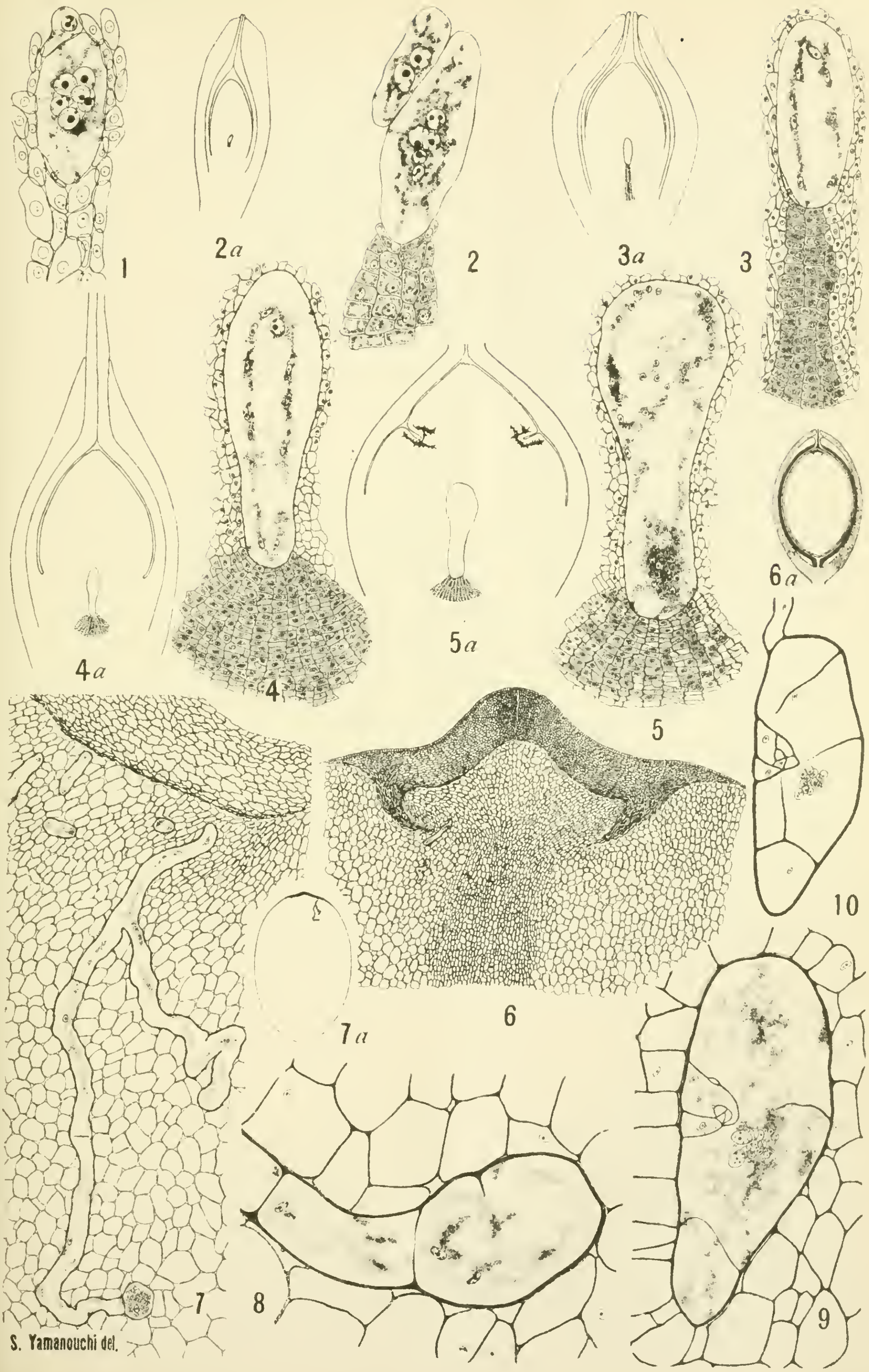
[6] LAND, W. J. G., Spermatogenesis and oogenesis in *Ephedra trifurca*. BOT. GAZETTE 38:1–18. *pls. 1–5.* 1904.

PLATE VII



S. Yamanouchi del.

FIG. 3.—Further development of pavement tissue; vacuolation of the sac. ×250.

FIG. 3*a*.—An ovule, showing general relation of regions shown in *fig. 3;* the tip of the nucellus has broken down (the only indication of a pollen chamber). ×40.

FIG. 4.—Further development of pavement tissue and embryo sac (probably fertilization stage). ×220.

FIG. 4*a*.—An ovule, showing relation of regions shown in *fig. 4.* ×40.

FIG. 5.—Later stage of embryo sac (possibly still in fertilization stage), with grouping of nuclei in the antipodal region, where tissue formation probably begins; beginning of disorganization of pavement tissue. ×210.

FIG. 5*a*.—An ovule, showing relation of regions shown in *fig. 5;* also the curious disorganization of cells and infolding of the epidermis in the nucellar beak. ×40.

FIG. 6.—Tip of seed; small amount of tissue of nucellar beak not destroyed by endosperm; differentiation of central and peripheral regions of endosperm, the former having advanced into the center of the nucellar beak and spread laterally, resulting in crushing nucellar tissue against the encroaching peripheral region of endosperm; sections of two suspensors shown. ×40.

FIG. 6*a*.—Mature seed invested by the fleshy "perianth;" outer integument differentiated into outer fleshy (white) and stony (black) layers; inner integument forming the micropylar tube; at apex of nucellus is indicated the remains of the nucellar tissue (shown with greater magnification in *fig. 6*). ×2.

FIG. 7.—A proembryo, showing the branching, suspensor-like elongation of the egg, with free nuclei and cleavage walls; also the embryonal cell containing a nucleus and numerous starch grains; sections of other suspensors shown, and also a small portion of the undestroyed tip of the nucellus. ×40.

FIG. 7*a*.—Outline to show the relation of parts illustrated by *fig. 7.* ×3.

FIG. 8.—Beginning of embryo formation by the embryonal cell, showing free nuclei and the beginning of a cleavage wall; a cleavage wall in the suspensor also shown. ×250.

FIG. 9.—Section of later stage of embryo, showing free nuclei, cleavage walls, and the beginning of small-celled tissue at one side. ×210.

FIG. 10.—An embryo reconstructed from serial sections, showing free nuclei cleavage walls, and the beginning of uninucleate cells. ×140.

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Compliments of R. R. Gates

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A STUDY OF REDUCTION IN *OENOTHERA RUBRINERVIS*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 111

(WITH PLATES I–III)

REGINALD RUGGLES GATES

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A STUDY OF REDUCTION IN *OENOTHERA RUBRINERVIS*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 111

REGINALD RUGGLES GATES

(WITH PLATES I–III)

The present contribution is a statement of some of the results obtained in the cytological study of *Oenothera Lamarckiana* and its mutants. Since these results have a more or less direct bearing on a wide range of fact and theory in various fields, their full discussion is reserved for a future time. The facts presented in this communication will be taken almost wholly from the study of *O. rubrinervis*, one of the most vigorous of the mutants. Other papers will be presented later, giving the further evidence upon which the conclusions of this paper rest, and attempting to indicate their bearing on the general problems of cytology and variation involved.

Material

The plants from which the material for these studies was obtained were grown from pedigreed seeds of DEVRIES, the purity of these cultures being further proven, in some cases, by carrying on the pedigree for another generation before collections were made. The results of these cultures, which are still being carried forward to later generations, will be published at another time in connection with an account of other studies on variation and hybridization in Oenothera. In this way it is hoped, if possible, to correlate the cytological data with the work in hybridization and variation. It seems to the writer that only by thus combining cytological with experimental studies is an explanation of the peculiar and remarkable phenomena of variation exhibited by the Oenotheras to be reached.

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The cytological studies presented here will be confined chiefly to the phenomena of synapsis and reduction in the pollen mother cell. Various forms have been studied, a complete series of stages being obtained in some forms and a partial series or only a few stages being examined in others. The forms investigated include (1) *O. rubrinervis*, (2) *O. Lamarckiana*, (3) *O. gigas*, (4) *O. nanella*, (5) *O. biennis cruciata*, a variety of the European *O. biennis*, (6) both *O. lata* (see **12**) and *O. Lamarckiana* from the F_1 of *O. lata*×*O. Lamarckiana*, and (7) plants resembling *O. gigas*, from the F_1 of *O. lata*×*O. gigas*. Preliminary reports have already been made upon some of these studies, in various connections (**11, 12, 13, 14, 15**). Reference will be made to some of these results later.

The material from each individual was collected separately in nearly all cases, in order to observe possible individual differences in the same race, either in the number of chromosomes or in other cytological features. I am indebted to Mr. C. H. SHATTUCK for making a number of these collections. The material for the study of *O. rubrinervis* was obtained from a number of individuals grown in two different seasons and representing several strains derived from the same original pedigree. Sections were cut from six of these, and it may be stated here that in *O. rubrinervis* no individual differences were discovered, either in the number of chromosomes, which was 14 in all cases, or in any other features. In some of the other mutants, also, a number of individuals were examined. It was found necessary to reserve the account of *O. gigas*, which presents several features of special interest, for a separate paper. A preliminary report on this form has already been made (**14, 15**).

For various reasons, *O. rubrinervis* was chosen as the most favorable form for a thorough study of synapsis and reduction. The nuclei and chromosomes of Oenothera are small, and for this reason the selection of the most favorable type for study is a matter of some importance. In *O. rubrinervis* the pollen mother cells, although they vary much in size, are usually considerably larger than in *O. Lamarckiana*, the nuclei being also proportionately larger. The reason for this will be explained later. The chromosome number being low in most of the forms ($2x=14$, $x=7$), they can be counted without any difficulty, notwithstanding their small size. Another notable advantage in comparing this with other studies in reduction is in the shape of the chromosomes, which are globular or somewhat oblong or cylindrical in most stages of mitosis, and are never greatly elongated or looped. For this reason it is a comparatively easy matter to obtain accurate counts of the chromosomes in the pollen mother cells of any of the forms. This shape is also found to be very advantageous in a study of the events of reduction following synapsis. The appearances are clear and easily interpreted, in striking contrast to the forms with long twisted chromosomes, such as have been made the basis of many of the studies on reduction in plants.

On the other hand, the somatic nuclei and chromosomes are very much smaller, and in metaphase the latter are elongated and looped, making it impossible to count them with the same degree of accuracy. Some of these appearances have already been described elsewhere (**12**, p. 19). Thus while it was found that the chromosomes could be counted almost equally well in pollen mother cells of all the forms studied, *O. rubrinervis* was found to be especially favorable for the investigation of reduction phenomena, especially the events of synapsis and the prophases of the heterotypic mitosis. The account given here will refer throughout to *O. rubrinervis*, with occasional comparisons with other forms. Later papers will take up these other forms in detail, in so far as this is necessary after the account presented here. Special attention will be given at that time, in particular, to the later stages, beginning with the telophase of the heterotypic mitosis, and also to the interesting conditions in some of the hybrids. The detailed account in *O. rubrinervis* will not be carried farther than the metaphase of the heterotypic mitosis, at which time the essential events have already taken place.

Methods

The usual methods of cytological technique were employed, various chrom-acetic and chrom-osmo-acetic solutions being tried until satisfactory fixation was obtained. The thickness of the sections varied from 4 to 10 μ. The latter thickness was found most favorable for counting chromosomes, because it is somewhat greater than the diameter of the nuclei, many of which in such sections were therefore uncut. It is possible to determine easily whether a nucleus has been cut by the knife by examining it in low and high focus. The chromosomes in such uncut nuclei can then be counted with absolute accuracy, either in the prophase of the heterotypic mitosis before the disappearance of the nuclear membrane, or in the telophase after the walls of the daughter nuclei are formed. In nearly every individual examined, large numbers of such cases, all yielding the same result, were counted before the number was finally determined upon. The chromosomes could also be counted in certain positions on the spindle, particularly in anaphases, but in metaphase they were usually too closely grouped to allow of satisfactory counting.

In the second division, particularly in the forms having seven chromosomes as the gametophytic number, the chromosomes could be counted with certainty in almost any stage of mitosis. The thinner sections were used chiefly in the study of spirem and synapsis stages, although here also the comparatively short length of the thickened spirem frequently made it advantageous to study uncut nuclei in which the spirem could be followed throughout its length.

Of the various stains Heidenhain's iron-hematoxylin was found to be superior for chromosome counting and for clear differentiation of chromatic structures in nearly all stages of synapsis and reduction, safranin-gentian being used occasionally for comparison or for differentiating particular cytoplasmic structures. Orange G was also used with the iron-alum stain for bringing out clearly certain special features, particularly the protoplasmic connections between mother cells, which will be described later.

Description

EARLY STAGES

Some of the very early stages of the anthers, previous to the formation of mother cells, have been studied particularly with the purpose of tracing the origin of the bodies which were called heterochromosomes in my first paper. The provisional use of the name was based on the very close resemblance of these bodies to the chromosomes in appearance, and their frequent presence close by, or in some cases apparently attached to, the heterotypic spindle. They were not stated to pass undivided into one of the daughter nuclei, as misquoted by TISCHLER (32), but to remain outside in the cytoplasm where they gradually disappear. The study of their early history shows that no line of distinction can be drawn between them and the large body readily recognized as the nucleolus. They are then smaller nucleoli, not differing essentially in origin from the single larger body which is almost constantly present in the mother cell during synapsis and prophase, but diverging from the latter somewhat in their later history.

In the earliest stages studied, the young meristematic cells of the anther primordia are very small (*figs. 1, 2*), and the tissues are wholly undifferentiated, except the epidermal layer. Usually several smaller nucleoli are present in each nucleus of the meristematic cells, in addition to the larger nucleolus. Compared with the cells of the anther wall when they are no longer meristematic, the smaller nucleoli of the former are about the size of the nucleoli of the latter, which are approximately equal in size. There is nothing in the latter corresponding to the larger nucleolus of the meristematic cells. Probably afterward one of these nucleoli enlarges as the cell increases in size, or it is possible that the nuclei of meristematic cells are always derived from previous ones which already possess a large nucleolus.

Chromatic staining bodies are also found closely appressed to the nuclear membrane in all the meristematic cells (*figs. 1, 2*). This tendency for chromatic material to accumulate on the nuclear walls gives these nuclei a characteristic appearance. These bodies often appear like a thickening of the membrane itself.

At the next stage studied many cell divisions have taken place, and the sporogenous, tapetal, and wall tissues have been differentiated. The sporogenous cells have increased enormously in size, and form a single row in longitudinal section down the center of the anther, the walls of these cells being especially thickened and distinct (*fig. 3*). The cells of the surrounding tapetal layer have also increased greatly in size and are distinctly marked off from the sporogenous row. In the sporogenous cells the nuclei (*fig. 4*), though much increased in size, have not increased in proportion to the cytoplasm. The large nucleolus, much larger than in the earlier stage, is now a conspicuous object in the nucleus. Smaller nucleolar bodies are almost invariably present, but masses are no longer found attached to the nuclear membrane. (The characteristic masses, however, may remain for some time attached to the nuclear walls of the tapetal cells).

Figs. 5–10 are from drawings of other nuclei at this stage of development. In the majority of cases one or two smaller nucleoli occur in addition to a single large one, but rarely (*fig. 6*) two large nucleoli of equal size may be found; and very frequently the number of small bodies, of equal or unequal size, may be greater, reaching as many as five or six. *Figs. 5, 7, 8, 9* show these in various stages of fusion with each other and with the large nucleolus.[1] They are thus not in any sense autonomous bodies. It appears that usually these fusions take place until only one large nucleolus and one or two smaller ones are present during synapsis and diakinesis. But occasionally the fusions do not take place, and several of these bodies may then be present in the later stages. The number of these nucleoli finally present depends, then, largely upon the amount of fusion which has previously taken place between them. In the later stages one large nucleolus is almost invariably present and usually a smaller one bearing a certain proportion to the larger in size, though the latter may vary in size and number as already stated. There is usually a clear area around the large nucleolus, as in the earlier stage, and threads of the reticulum may or may not cross this and appear to be attached to the nucleolus (*fig. 4*). The reticulum of the cytoplasm usually stains rather more deeply at this time than that of the nucleus. It may as well be stated at this time that in the resting nuclei of the pollen tetrad and in the nuclei of the nearly mature pollen grains of Oenothera one finds (*fig. 11*) the same condition of the nucleoli as in the mother cells, namely, usually one large and one small nucleolus bearing a rather definite size relation to each other, with sometimes additional small ones.

The sporogenous rows are differentiated from the tapetum by the greater growth of the cells, nuclei, and nucleoli of the former. At the same time they are distinctly marked off by the formation of a continuous thickened wall between tapetum and archesporium (*fig. 3*). It is obvious that as the cells and nuclei increase in size, the nucleolus grows also. Up to the time of synapsis the mother cells usually form a compact tissue, but about this time the cells begin to break apart at the corners where they meet, and before diakinesis is reached they are completely rounded off and independent, or they frequently remain connected with other mother cells only at the ends. In the meantime the cavity of the loculus grows rapidly, so that the mother cells, in normal development, usually lie loose in the cavity.

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[1] Miss NICHOLS (**21**) figures what are in all probability stages of fusion of large and small nucleoli in Sarracenia pollen mother cells, but interprets them as a budding-off of small bodies from the nucleolus. The budlike attachments to the nucleolus frequently observed by other authors are doubtless to be explained in like manner.

The events of synapsis and reduction usually go forward simultaneously throughout a flower, with comparatively little variation in the different parts of the same loculus or in the different anthers of a flower. In one flower, however, wide variation was found in different anthers, but comparative constancy in the loculus. One anther of this flower was in synapsis, another in diakinesis, another in metaphase of the heterotypic mitosis, and in still another some of the mother cells had completed the second mitosis. No abnormalities in the cytological condition of this flower were observed.

SYNAPSIS

After the stage described in *fig. 4*, the nucleus increases greatly in size, but without an appreciable increase in the size of the cell. The single row of sporogenous cells divides, so that there are usually two rows of pollen mother cells. Occasionally three or more mother cells are found in the cross-section of a loculus. In general there are fewer divisions than in the other forms, and this is at least one of the reasons why the mother cells are on the average larger than, for example, in *O. Lamarckiana*.

The resting nucleus of the pollen mother cell increases in size and begins to show signs of approaching synapsis. *Figs. 12, 13, 14* show stages in the beginning of this process. A number of these stages were found—although they are uncommon—in the same sections with regular synapsis stages. In some cases they occurred side by side with mother cells in which the synaptic knot had already been formed. A complete series of stages may be found in the same section, from the beginning of contraction to the formation of a close synaptic ball. The cytoplasm in these cells shows no contraction whatever, but is perfectly fixed. For this and other reasons there can be no doubt that this is a real contraction stage, leading to synapsis, and not a result of imperfect fixation, as one might judge on first examination.

That these nuclei are going into synapsis and not coming out is shown by several features: (1) the extremely delicate character of the threads, like those of the resting nucleus; (2) the fact that the periphery of the reticulum as it contracts frequently preserves perfectly the curved outline of the nuclear wall (*fig. 12*); (3) immediately after synapsis the thread is somewhat shorter and thicker than previously and appears to be continuous, while in the earlier contraction stages we still have the appearance of a reticulum (*fig. 13*).[2] As the contraction progresses, the threads are gradually rearranged from an anastomosing reticulum to a very long and continuous delicate thread. The exact manner of this rearrangement could not be observed, but one finds many transitions (*fig. 14*) from the anastomosing reticulum of the resting nucleus to the closely coiled and apparently continuous spirem of the synaptic knot (*fig. 15*). The contraction may take place from one side of the nucleus, leaving the reticulum attached for a time to the nuclear membrane at one point (*fig. 13*), or it may take place simultaneously from all sides (*fig. 12*). A few threads of the reticulum usually remain attached for a time to the nuclear membrane while the contraction is going on. These are drawn in finally as the synaptic ball becomes more compact.

The small number of these intermediate stages found indicates that they are passed through rather rapidly, the frequency of the occurrence of synapsis stages indicating, on the other hand, that this condition is of considerable duration.

No indication of a doubling or pairing of the threads during these intermediate contraction stages could be observed, though they were carefully searched for. Moreover, in the earliest stages of the synaptic ball the thread appears to be as thin and delicate as in the reticulum, which does not favor the view that a pairing has taken place. The evidence, then, so far as it goes, is decidedly not in favor of a pairing.

During these stages the nuclear membrane is often indistinct, making it difficult to define accurately the limits of the nucleus. The same condition is observed during synapsis, which is found in the same sections. In places the nuclear membrane has either disappeared or is too delicate to be observed. The cytoplasm, however, retains the original outline of the nucleus. Mottier (**20**) has apparently observed similar conditions of the nuclear membrane at this time. In some cases it is ruptured and a portion of it is actually carried inward with the nuclear reticulum at the beginning of the contraction (*fig. 12*). One is tempted to explain this as an artifact; but that this is not the explanation is shown by the considerations already mentioned. The explanation appears to be that as contraction proceeds a portion of the nuclear membrane may be torn away and carried inward attached to the threads. Frequently in these stages one finds the nuclear membrane present on one side of the nucleus but invisible elsewhere. This is the case in *fig. 12*, although the membrane was drawn as though complete. Observations of other nuclei bear out this interpretation, the nuclear membrane being clearly visible in some cases attached to portions of the reticulum which have contracted far away from the original position of the nuclear wall. In the late prophase, when the definitive chromosomes are formed, a distinct and perfect nuclear membrane is invariably present, so it would appear that in such cases as those just described a new membrane is afterward formed.

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[2] This explanation assumes, of course, that the synapsis stages themselves are normal and not due to artifact, as I presume all cytologists will now agree, although SCHAFFNER (29) apparently still entertains some doubt on the subject. The regularly coiled arrangement of the thread in the synaptic ball appears to me to be one of the best arguments against this stage being an artifact. Evidently a rearrangement of the threads is going on as contraction proceeds.

Mention must now be made of the chromatic staining material of the nucleus during these stages. The nucleolus is frequently, though not always, included within the synaptic knot. There is a tendency for other dark-staining bodies to accumulate near the periphery of the nucleus (*figs. 12, 14*); as contraction proceeds these are swept in by the reticulum. The exact relation they bear to the threads is not known. In some cases they appear, in the later stages of contraction at least, to form a part of the threads themselves, in other cases they appear to be merely inclusions in its coils. These bodies show no constancy in number, size, or shape. As the spirem takes on the appearance of the synaptic knot, they are still found in its meshes, and portions of the thread itself may also stain darkly, suggesting a solution of a part of their substance and its transfer into the thread. Even when the greater part of the spirem is completely decolorized certain portions of it retain the stain. This appears to be partly due to the denser aggregation of the spirem in these regions, but in some cases it is evidently due to the presence of bodies which retain the stain and appear to be giving up the stainable part of their substance to the spirem. These bodies are evidently not the prochromosomes found by OVERTON (**22**) in certain dicotyledons, nor are they the gamosomes of STRASBURGER (**30, 31**).

Just the relation these bodies sustain to the spirem is not easy to determine. From *figs. 12* and *14* it is evident that they are at first small "nucleoli" caught in the contracting reticulum, but quite independent of it. Later they appear to give up a portion at least of their material to the spirem, finally disappearing as independent bodies. Usually, however, at least one of these bodies remains independent, and appears in synapsis and diakinesis as a small nucleolus bearing a definite relation to the size of the large nucleolus, being about the size of a chromosome. These bodies are usually free in the nuclear cavity (*fig. 15*). A certain depth of stain is required for demonstrating them during synapsis, for they usually decolorize more quickly than the large nucleolus. With a favorable stain they are found to be of strikingly uniform occurrence at this time. A plasma stain such as orange G may be used with advantage to demonstrate their presence. The uniformity in their occurrence is so great that for some time they were thought to be constant in size and number. With the demonstration of their inconstancy and their origin we have chosen to call them merely small nucleoli, as there appears to be no sufficient reason for another name. The (large) nucleolus disappears with great promptness immediately after the nuclear membrane breaks down, only persisting for a time in a few rare instances. In no case has fragmentation of the nucleolus, previous to its disappearance, been observed, although the presence of deeply staining globular bodies occasionally found near the periphery of the cytoplasm might be accounted for in this way. The mass of the latter, however, is sometimes greater than that of the nucleoli. The smaller nucleoli persist and are frequently found close by the heterotypic spindle. They may also be found on the homotypic spindle (*fig. 41*). Apparently they never reenter a nucleus, but remain in the cytoplasm until they finally disappear. These bodies have been found showing the same behavior in all the forms studied.

POST-SYNAPTIC STAGES

Synapsis lasts for a comparatively long time, as shown by the frequency of its occurrence in the material sectioned. During this time the spirem shortens and thickens and then begins to arrange itself more loosely in the nuclear cavity. This shortening and thickening is progressive (*figs. 16–18*) and apparently continues for some time. During these stages the thickness of the spirem may be nearly uniform throughout, or it may vary greatly, giving a moniliform appearance, or the spirem may appear irregularly constricted at varying intervals. In other cases, with a certain depth of stain it is seen to be composed of lighter and darker areas more or less regularly alternating. Portions of the thread may appear homogeneous or may show the lighter and darker areas, according to the depth of stain (*fig. 17*). In more deeply stained nuclei, such as *fig. 16*, the thread appears homogeneous throughout. These darker areas are the chromatin discs or chromomeres of various authors; and they give the thread a very characteristic appearance. During this well-defined stage the greatly thickened spirem is loosely distributed in the nuclear cavity. Deeply staining bodies still appear attached to or enmeshed in the coils of the thread.

At this time one finds undoubted indications of parallel threads. When represented by camera drawings in one plane the evidence for this is not so satisfactory as in the original preparation, but there is no doubt of their occurrence. As already stated, in the earlier stages previous to and during synapsis, parallel threads could not be observed, and it has not been determined whether they were really absent or whether the failure to observe them was due to their extreme delicacy. Hence it cannot now be stated whether they have arisen through an approximation of spirems at an earlier period, or through a split in the single continuous spirem. This matter will be discussed later.

Following this stage a second well-marked contraction takes place (*figs. 18, 20, 21*), apparently quite as typical and constant in its occurrence as the first contraction stage, which is ordinarily identified as synapsis. This contraction is of much shorter duration, however, and entirely different in appearance, owing to changes which the thread has undergone since synapsis, resulting in a great amount of shortening and thickening of the spirem. MOTTIER (**20**) has recognized this second contraction stage in Podophyllum, Lilium, and

Tradescantia, though he formerly thought it resulted from bad fixation; and it appears to have been observed also by FARMER and SHOVE (**10**). MOTTIER states that in these forms there is but little shortening of the spirem between synapsis and segmentation into chromosomes. In Oenothera, on the contrary, as is evident from a comparison of *figs. 15* or *16* with *22*, a very considerable amount of shortening as well as thickening of the spirem takes place during this interval. During the second contraction the paired threads apparently fuse, and further shortening of the (from now single) thread results in an enormous amount of thickening of the spirem, so that when it uncoils from this second contraction it has approximately the thickness of a chromosome and exhibits only a few loops. It can then frequently be traced through nearly its whole length. At this time there is a great amount of variation in the thickness of different parts of the spirem, as seen in *figs. 22* and *23*. *Fig. 19* is a portion of the spirem at this period, drawn with a higher magnification. It shows the chromatic bodies, which vary in size, imbedded in the linin substratum. As to how far two different substances are represented, I am at present unprepared to say.

DIAKINESIS

The single thick thread now segments transversely into 14 chromosomes, the sporophyte or 2*x* number. At this time there is no indication whatever of a longitudinal split in the thread. Even when greatly washed out, the material of the chromosomes appears perfectly homogeneous, or if a granular structure is observable there is in its arrangement no indication of the previous split. At the time of this second contraction a pair of chromosomes is frequently observed separated from the spirem and apparently always lying with their long axes parallel and connected at one end (*figs. 20, 22*). This condition occurs very commonly, although in other cases the spirem is continuous throughout (*fig. 21*). In no case has more than one pair of chromosomes been observed to be thus precociously cut off in *O. rubrinervis*, though two such pairs have been observed in *O. lata* (see **11**, *fig. 19*). In no case has a single chromosome been observed to be cut off in this manner, and apparently they are invariably cut off in pairs, that is, bivalent chromosomes are detached.

What significance this early separation of chromosome pairs may have is not known, but it appears that the later history of these pairs on the spindle can be traced. In the paper just cited (**11**), the writer wrongly identified them with the smaller nucleoli which persist by the heterotypic spindle. These chromosome pairs are frequently so closely approximated at the end opposite the end of actual connection as to give the appearance of a ring. It was thought that these rings by condensation (which actually takes place) were reduced to the size of these nucleolar bodies. The latter had the size and shape of chromosomes, and with a certain depth of stain invariably appeared hollow. These pairs are not condensed to rings, however, but to chromosome pairs of the ordinary Oenothera type.

The spirem at this time varies greatly in thickness in different parts, exhibiting constrictions and dilatations which indicate more or less clearly where segmentation into chromosomes will take place. This segmentation may happen while the spirem is still in the contracted condition (*fig. 25*), or after it has again uncoiled and distributed itself in the nuclear cavity (*figs. 24, 26, 28*), or before this uncoiling is completed. The segmentation appears to be in some cases nearly simultaneous (*fig. 24*); in other cases the segmentation is successive, as in *fig. 23*, where the spirem is clearly divided into three portions and the constrictions for the formation of the chromosomes are so far advanced that the number of chromosomes to be formed by each segment can already be foretold with practical certainty. The segmentation at this time is into 14 chromosomes, the sporophyte number. A large number of counts made at this time demonstrate the absolute constancy of this number in all the individuals of *O. rubrinervis* examined. It is possible, however, that individuals of this race may be found whose chromosome number differs from this number by one. This matter will be discussed later.

In every single case where the count could be determined with certainty it was shown to be 14. These counts were all made from sections 10 μ thick, and from nuclei which were uncut by the knife. The less numerous counts made in the multipolar stage of the heterotypic spindle gave invariably the same number. In this case all in a given cell were obtained by examining the adjacent sections. In all, hundreds of counts were made. In such nuclei as *figs. 26, 29, 30, 31* there can be no possible doubt of the number of chromosomes present.

As already shown (*fig. 20*), one or in some cases more pairs of chromosomes may be cut off from the spirem before it undergoes segmentation, and frequently while it is still in the second contraction period. The exact method of origin of these pairs has not been observed, but they invariably, so far as observed, lie with their long axes parallel and connected at one end, from which it would appear that they were successive chromosomes on the spirem. In later stages, when the spirem has constricted into a chain of chromosomes arranged near the periphery of the nucleus, one or more pairs of chromosomes are found separated from the rest. Some of these have doubtless had the origin shown in *fig. 20*. Others appear to have originated later, as indicated in some of the figures, by successive chromosomes on the chain swinging around parallel to each other and thus pairing. Usually in diakinesis one or two such pairs are found, though occasionally there is no evidence of pairing. The highest number of pairs observed at this stage was five, with indications of pairing among the others (*fig. 29*); which is unusual. Later, in the multipolar spindle stage two distinct pairs are usually found in varying stages of conjugation (*figs. 35, 36*). A single case was observed (*fig. 37*) in which the fourteen chromosomes were all paired.

As the figures indicate, constriction of the spirem at regular intervals proceeds progressively until a chain of chromosomes is formed. When this has taken place, the chromosomes are at first several times longer than broad, and their margins have a very irregular, sinuous outline, like that of the spirem just previous to segmentation. They are not so long, however, that they can be twisted and looped in the confusing manner of many heterotypic chromosomes of plants. This is very gratifying in the study of these stages, since it permits a clearness of interpretation which would otherwise be unattainable. *Figs. 22* and *23* show the beginning of contraction, which has proceeded farther in *fig. 24*, leaving only the so-called linin connection between the chromosomes. The constrictions are all equivalent and the spirem thus segments into the sporophyte number of chromosomes and not into the reduced number of chromosome pairs. If successive chromosomes on the spirem are really the members of a pair, there is nothing in the manner of segmentation of the spirem to indicate this. However, it is clear enough that one chromosome frequently swings around, as already mentioned, and pairs with its neighbor on the spirem. We do not really have, then, a transverse division of chromosome bivalents, but a separation of whole (somatic) chromosomes. Nothing has been found in the earlier stages which would correspond to the gamosomes and zygosomes of STRASBURGER, and even should a pairing of parallel threads during synapsis occur (a possibility which will be discussed later), the final pairing is between chromosome bodies which were lying end to end on a single spirem thread.

The linin connections during diakinesis appear to be merely the more finely drawn out portion of the spirem between the chromosomes. As condensation and contraction of the chromosomes progress, these linin connections become longer and more delicate (*figs. 31, 33*). The chromosomes become more dense and compact, being at first oblong-cylindrical (*figs. 24, 26*) and then more nearly globular or pear-shaped (*fig. 31*). Certain chromosomes sometimes undergo this contraction more quickly than others, as in *fig. 29*, and the different stages of this condensation may occasionally all be found in the same nucleus. In other cases the globular appearance is due to the position in which certain chromosomes happen to be lying (*fig. 34*).

HETEROTYPIC MITOSIS

During the prophase stages last outlined the cytoplasm usually possesses a more or less obscurely radiate appearance. A felt-work of fibrillae finally appears around the nuclear membrane. Later these fibrillae come to run tangentially to the latter, terminating in the cytoplasm, and by their aggregation in certain regions the multipolar spind'e is formed. From this stage the fibers are rearranged to form the bipolar spindle, passing through conditions in which the spindle appears quadripolar or tripolar in section. In the meantime the nuclear membrane has dissolved and the chromosomes are found at first in a cavity surrounded by fibers which preserve the outline of the nuclear wall. Later they come in and become attached to the chromosomes. Usually the large nucleolus has vanished before this time, but occasionally it may still be seen (*fig. 35*). In *fig. 37* the small nucleolus is shown, which can very frequently be seen at this time. *Figs. 36* and *37* are merely sketches of the spindle fibers to indicate their general direction. *Fig. 35* is an unusual case. A cone of fibers appears to have been formed on one side only of the nucleus. The fibers are coming in and finding attachment to the chromosomes. The large nucleolus is still present, as well as two smaller ones.

The most critical stages of reduction have now been described and the remaining stages will be taken up with less detail at this time, but will be presented in full in a later paper. The chromosomes are at first irregularly arranged on the heterotypic spindle. As already seen, during spindle formation many of the chromosomes are frequently separate and unpaired. The attraction between the chromosomes which leads to pairing is evidently weak, so that it is doubtful if any pairing takes place at metaphase between chromosomes which had not previously paired. On the other hand, chromosomes which have once paired, no matter how early, appear to remain together until their separation in the metaphase of the heterotypic mitosis. Hence probably in many cases the chromosomes pass to the poles of the heterotypic spindle without having previously paired with each other, that is, they were merely lying loosely in the equatorial region of the spindle in metaphase, so that it was largely a matter of chance which pole any particular chromosome went to. This is believed to be a matter of prime importance in determining the final result of the reduction divisions in Oenothera, and the nature of the distribution of chromatin elements which takes place. Its possible significance will be pointed out in the discussion. *Fig. 38* shows the chromosomes just being drawn into the equatorial plate of the heterotypic spindle. In the examination of thousands of spindles in about this stage, one usually finds the chromosomes spread out in several planes along the long axis of the spindle. Of course some of these are early anaphase stages in which the chromosomes have begun their journey to the poles, but the condition is seldom found where the chromosomes are arranged regularly in pairs on the spindle. The daughter chromosomes seldom advance toward the pole in a single plane, as is the case in so many forms, but are more or less irregularly strung out along the spindle in their passage to the poles. This is in striking contrast with their behavior in the homotypic mitosis.

Usually in the early anaphase of the heterotypic mitosis a longitudinal split appears in the daughter chromosomes. This split does not stop short of one end, giving a V-shaped body as in many plant chromosomes, but usually passes right through, forming two independent bodies, which, however, remain paired in the telophase and occupy a great variety of positions in regard to each other. The homotypic chromosomes thus assume many of the characteristic shapes which are usually observed in the heterotypic chromosomes of other forms, such as X, Y, V, H, etc. The failure of the heterotypic bivalents to form these shapes is due partly to the weaker attraction between the members of a pair, but largely to a difference in their shape, each member of a pair being usually more rounded in the heterotypic and more elongated and rodlike during the stages between the two mitoses.

The telophase of the heterotypic mitosis is one of the best stages for counting the chromosomes, as they are distributed at equal intervals around the periphery of the nucleus, no two ever being in contact and the halves of each (bivalent) chromosome rarely separating. The chromosomes now evidently repel each other, while the halves of each chromosome attract each other rather strongly. The halves of these bivalent chromosomes are usually short rods, but they may be dumb-bell or hour-glass shaped, or nearly globular, as previously mentioned (**12**). Sometimes, however, this split fails completely to occur in the anaphase, the daughter chromosomes remaining single and globular or somewhat elongated (*fig. 39*). These telophase stages and the prophases of the homotypic mitosis will be taken up in detail in a paper dealing with different forms. These results, therefore, will not be duplicated here, but a brief statement of the events of the second mitosis will be given.

HOMOTYPIC MITOSIS

In the telophase of the heterotypic mitosis the nuclei never pass into the resting condition and the chromosomes never lose their identity completely, though they spread out and anastomose with each other more or less. Nucleoli are formed, as previously described (**11**). These stages between the two mitoses last for some time, but the events of the second mitosis are passed through very quickly. The two homotypic spindles are formed simultaneously and their axes are at various angles to each other. Spindle formation is the same as for the heterotypic mitosis, except that the spindles are smaller. In regard to the chromatin, suffice it at present to say that the chromosomes of the homotypic prophase show the same general types and are often identical in appearance with those of the heterotypic telophase. There can be no doubt that the bivalent bodies which appear on the homotypic spindle are the same bodies that were present in the telophase of the heterotypic. *Fig. 41* shows an early anaphase of the second mitosis, the members of each pair having just separated. One of the small nucleoli appears by one of the spindles.

IRREGULARITIES

In *fig. 39* spindle fibers are seen in the cytoplasm by the side of the spindle in anaphase. This may be connected with a condition which is illustrated in *fig. 40*. Six such cases were observed in which a regular spindle occurred at the side of the mother cell instead of between the daughter nuclei, after the partial or complete disappearance of the heterotypic spindle. Some of these cases were in the telophase of the heterotypic spindle (*fig. 40*); others were in the prophase of the homotypic. In these cases the spindles were regularly formed and rather sharp-pointed and occupied the same position at the side of the cell; of course they contained no chromosomes. The method of their origin is unknown, but it seems probable that they are connected with the condition observed in *fig. 39*. Mother cells which probably indicate an intermediate condition, in which irregularly arranged fibers were found at the side of the cell, were occasionally observed. They may merely indicate a persistence of the kinoplasm of the heterotypic spindle after its function has ceased, but their structure appeared remarkably definite in most of the cases observed.

A single case of extra nuclei in the pollen tetrad was observed in *O. rubrinervis*. These have been previously described in *O. lata* (11), where they are common occurrences in connection with pollen degeneration. The single case observed in *O. rubrinervis* is sketched in *fig. 42*. Two small nuclei are present in addition to the four larger ones composing the tetrad. The nuclei had passed too far into the resting condition to count the chromosomes in each nucleus.

POLLEN DEGENERATION

The general question of pollen degeneration in Oenothera is an interesting one. It reaches its extreme expression in *O. lata*, which is usually completely sterile in this regard, and in which I have already shown (**11**) that irregularities occur during the reduction divisions similar to those found in sterile hybrids. The question of sterility is evidently, as Tischler (**32**) suggests, a relative one.

In *O. rubrinervis* one is led from a gross examination to judge that the pollen production is copious and probably equal to that of *O. Lamarckiana* itself, but in reality many of the pollen mother cells fail to complete their divisions. From an examination of sections of anthers of *O. rubrinervis* it is found that in some loculi a large number or perhaps nearly all the mother cells may be degenerating in the synapsis stage. Frequently the cells are flattened and distorted, appearing pressed together for lack of space in the loculus. The chromatic contents of such cells often form a dense irregular mass, or their nuclei may be in normal synapsis or mitosis, notwithstanding the distorted shape of the cell; while still other cells of the same loculus may be entirely normal. Even earlier, in the archesporial stage, the tapetal cells in many sections were found to be breaking down, as in *O. lata* (**11**). No indications of degeneration have yet been observed in mother cells of *O. Lamarckiana*, and very few in the tapetum.

The percentage of mother cells which thus degenerate in *O. rubrinervis* was not determined. Tischler (**32**) suggests that the causes of sterility in mutants are the same as those in hybrids and in plants under cultivation. This general cause he designates as a disturbance or derangement of the constitution of the idioplasm, which he thinks has taken place in the production of mutants as well as in hybrids and under the conditions of cultivation.

PROTOPLASMIC CONNECTIONS

It is an interesting fact that large and rather conspicuous protoplasmic connections are found between the pollen mother cells in *O. rubrinervis*. They are usually quite easily seen and it is probable that they are always present. They consist of delicate strings or threads of cytoplasm connecting adjacent mother cells. In size they vary greatly, from the delicacy of a spindle fiber to a coarse thread or strand connecting the cells (*figs. 45, 46*). When the cytoplasm has shrunken slightly away from the cell wall they are particularly clearly observable. These connections appear to be in all cases between mother cells, and in no case have they been observed between the mother cells and the tapetum. Generally one such strand is seen connecting two cells, but not infrequently there are two or three or occasionally even more. There is no constriction or change in the nature of the connective as it passes through the cell wall. These connections are even larger and more conspicuous in *O. gigas*, where the mother cells are also much larger. They have not been observed in *O. Lamarckiana* or the other forms, but they doubtless occur in all, being probably smaller and more inconspicuous in some.

Discussion

The method of reduction described in this paper at once raises a number of questions of prime importance from the cytological standpoint, as well as from that of the relation subsisting between hereditary and cytological phenomena. A discussion of all these features will not be attempted at this time, the intention of the writer being merely to indicate the general directions in which the facts point and the possible bearing which these data may be found to have on the problems connected with the phenomena of mutation in Oenothera. A fuller discussion of these subjects is reserved for a future time, after the presentation of further data. In the present communication reference will be made only to the most recent papers on reduction in plants, the purpose not being a review of the literature, or a discussion of present views, except in so far as they bear directly on the matter in hand.

The recent accounts of reduction in plants, given by BERGHS (3, 4, 5, 6), GRÉGOIRE (16), STRASBURGER (31), ALLEN (1, 2), MIYAKE (18), OVERTON (22), ROSENBERG (25), YAMANOUCHI (33), and others, have agreed in so far as the following general course of events is concerned: In synapsis a pairing of homologous maternal and paternal elements occurs either in the form of gamosomes (STRASBURGER and MIYAKE), prochromosomes (OVERTON), or parallel threads (ALLEN, ROSENBERG, GRÉGOIRE, BERGHS, CARDIFF 7, and YAMANOUCHI). In every case two parallel threads result, which unite more or less intimately about the time of synapsis or later. After the events of synapsis, a longitudinal split reappears in the thickened spirem threads, this split representing the line of approximation of the two original spirems. Transverse segmentation into pairs of chromosomes, which are believed to be homologous somatic chromosomes of maternal and paternal origin, then takes place. The halves of these bivalent chromosomes, which lie side by side, are then distributed in the heterotypic mitosis, which is thus a reduction division. In the anaphase of the heterotypic mitosis a longitudinal split appears in the daughter chromosomes, which is regarded as a premature split for the homotypic mitosis, the latter being thus an equation division. The persistency with which this general account has been given, notwithstanding differences in detail, particularly preceding and during synapsis, leads the writer to the belief that it is probably correct in its main outlines, at least in many of the forms described. This being judged to be the case, every effort was made to bring the account in Oenothera into harmony with this general course of events but without success, for Oenothera is found to deviate in some important particulars, as is already evident from the description.

Another general account of reduction in plants, which was adhered to by STRASBURGER as late as 1904 (**30**), and has been held notably by FARMER and MOORE (**8, 9**), FARMER and SHOVE (**10**), SCHAFFNER (**28**), MOTTIER (**19, 20**), and others, to mention only a few of the recent papers, is in general as follows: The split in the spirem which occurs at about the time of synapsis is a true split, such as may occur in the prophase of somatic mitoses, and is not preceded by a pairing of parallel threads, but the thread is single from the beginning. This split afterward closes up as the thread shortens and thickens after synapsis, and the single spirem so formed segments usually into the reduced number of chromosomes, which are thus arranged successively end to end. Each such bivalent chromosome thus consists of two halves arranged end to end, not side by side, and the heterotypic mitosis thus separates successive whole chromosomes on the spirem, being therefore, as in the other account, a reduction division. The split which appears in the anaphase of this mitosis is interpreted as a reappearance of the earlier longitudinal split of the spirem. The homotypic mitosis is therefore an equation or longitudinal division.

There are of course minor differences in these accounts, SCHAFFNER (28) stating, for example, that in *Lilium tigrinum* there is a splitting of granules in the spirem, but the linin thread remains single. Differences of opinion are also expressed regarding the arrangement of the loops of the spirem before segmentation, and their relation to the chromosomes formed.

These two general schemes agree that the heterotypic mitosis is a reduction division separating whole somatic chromosomes, while the second division is longitudinal. The essence of the distinction is that the first view regards the chromosome bivalents as formed by a side-by-side union of homologous chromosomes through the medium of parallel threads, while the second view holds to an end-to-end union. It will be seen that, omitting the points which are left undetermined, the account in Oenothera corresponds more nearly with the latter scheme than with the former, though differing in some respects from both. ROSENBERG (25), from a comparison of forms having long and short chromosomes, has attempted to harmonize the latter view with the former. He examined Listera, Tanacetum, Drosera, and Arum, and found that, for example in Drosera, which has short definitive chromosomes much like those of Oenothera, the spirem first segmented into long twisted chromosomes lying in pairs with their long axes parallel. Later, as they condensed into the short, rounded definitive chromosomes, they frequently swung around end to end, so that an observer seeing only the later stage would conclude that they had been arranged tandem on the spirem at the time of their origin. Similar conditions were sometimes observed in Listera. I think my *figs. 22–28* make it evident that this explanation will not apply to Oenothera. The chromosomes in Oenothera do not undergo any such great amount of condensation, but are already thick, heavy bodies when first formed from segmentation of the spirem (*fig. 24*). Their diameter at this time is about the same as that of the spirem just previous to segmentation, as is shown by comparing *figs. 22* and *23* with *figs. 24* and *26*. The fact that as many as eight or more chromosomes may be found forming a single connected chain (*fig. 26*) also renders this explanation impossible.

MIYAKE (18) finds that after the pairing of elements in synapsis (the exact method of this pairing need not be entered into here) in

Galtonia and Tradescantia, a longitudinal split appears in the thickened thread, and the double spirem thus formed breaks transversely into the reduced number of chromosome pairs. Later, in these forms, a secondary union between the chromosomes is claimed to take place, forming a single connected chain of chromosomes (as in Oenothera). Sometimes a pair of chromosomes lies free by itself at this time. Then by further shortening the chromosomes of Galtonia again fall apart into pairs, though in Tradescantia they frequently remain connected even after spindle formation. The apparent similarity of the chromosome chain thus described by MIYAKE in Galtonia to the condition in Oenothera, led the writer to make an endeavor to harmonize the two accounts. But instead of this, all the evidence obtained from a critical study of the stages concerned shows that in Oenothera a single very thick spirem breaks transversely into the sporophyte number of chromosomes. A critical examination of *figs. 22–28* will make it clear, I think, that we are following the progressive segmentation of a single spirem, and there is no room for stages between, in which a double spirem breaks into two parallel series of chromosomes. Moreover, it is hardly likely that secondary fusions between chromosomes would take place to such an extent as is shown in *figs. 23* and *24*. In nuclei such as *fig. 20*, in which a pair of chromosomes is cut off prematurely from the spirem while still in the second contraction, they are invariably connected at one end and rarely, if ever, at the other (though sometimes the close approximation of the latter ends may give the false appearance of a ring). This would not be the case if they came from separate paired threads merely lying side by side, so that this connection shows them to have been really successive on the spirem. From this evidence the writer cannot see how anything except a distortion of the facts can lead to the assumption in Oenothera of two parallel threads breaking into chromosomes. Hence the conclusion is that the double threads appearing in the stage represented by *fig. 17* have united to form a single thread, which then breaks transversely into the sporophyte number of chromosomes.

This corresponds fairly well with STRASBURGER'S 1904 (**30**) account of the post-synaptic stages in Galtonia, and suggests to the writer that perhaps after all the earlier account may be nearer the facts, so far as the points here under discussion are concerned, than the paper of 1905 (**31**). The close similarity of the conditions in Galtonia and Tradescantia during diakinesis to those in Oenothera suggests that they may be found finally to conform to Oenothera in these later stages. Whether or not this will be found to be the case, we must conclude that in Oenothera the longitudinal fission in the spirem (however it originated) closes up, and that after the second contraction, or during it, the thick thread segments into the sporophyte number of chromosomes. Since this diverges in important respects from nearly all the recent accounts of reduction in plants, the conclusion is that reduction probably takes place differently in different plants. Whether or not the results are different from the standpoint of a qualitative distribution will not be discussed now. The writer believes the above conclusions to be necessary, despite the fact that authors have reached different conclusions in regard to the same plant, particularly in such cases as Lilium and Podophyllum.

The next important point which requires discussion and which was left undecided in the statement of observations, is in regard to whether the double thread observed after synapsis arises from an approximation of parallel filaments or through a primary split in the thread. It may be well to examine the results which follow from either assumption. The writer hopes later to determine more definitely this difficult matter. On the first assumption of a lateral approximation in synapsis of two spirems representing respectively the maternal and paternal chromosomes, we should expect the double thread so formed to segment into the *reduced* number of chromosome pairs, in order to conform to the current account in forms in which there is a pairing of spirems, for example ALLEN (**1**), GRÉGOIRE (**16**), and YAMANOUCHI (**33**). Instead, however, the spirem segments into the unreduced number of bodies. We may still assume that each of these bodies consists of maternal and paternal longitudinal halves still closely held together and resulting from a previous approximation. According to this view the first mitosis would separate bodies which were arranged successively on the spirem, while the second mitosis would separate the maternal and paternal halves of these bodies. The reason for such a result would be that the maternal and paternal spirems remained closely fused after pairing, so that their elements were separated in the second mitosis instead of the first. This view is scarcely admissible for several reasons. In the first place, on this hypothesis transverse segmentation of the spirem must have taken place not only between the (bivalent) chromosomes but also in the middle of each chromosome, in order to give a chain of fourteen bodies. Such a segmentation seems unlikely. Another possible explanation would be that the chromosomes have lost their identity during synapsis, and that the bodies we are dealing with now are new arrangements of the chromatic material, irrespective of the somatic chromosomes. Many considerations, however, strongly support the belief that these bodies really represent the somatic chromosomes. The facts so far educed in Oenothera, in the opinion of the writer, all favor the hypothesis of the separate existence and genetic continuity of the chromosomes from one generation to another. In this connection may be cited certain plants from the F_1 of *O. lata* × *O. gigas*, which as stated elsewhere (**14**) have 21 chromosomes as somatic number, 10 of which regularly go to one pole of the heterotypic spindle and 11 to the other. Occasionally, however, the segregated numbers of chromosomes are 12 and 9, one chromosome having gone to the wrong pole of the spindle. In this hybrid 7 of the chromosomes are maternal and 14 paternal. If in this case there were a pairing of maternal and paternal spirems, it is difficult to see how it could be accomplished and result in the distribution of chromosomes in the heterotypic mitosis already stated.

It will be instructive to compare the chromosome history in this cross with the often-quoted condition found by ROSENBERG (**23, 24**) in *Drosera longifolia* × *D. rotundifolia*. *D. rotundifolia* has 10 chromosomes and *D. longifolia* 20, as the gametophyte number. The hybrid naturally has 30 chromosomes in its sporophyte tissues, but in diakinesis 20 chromosome bodies appear, 10 of which are double, consisting of a larger and a smaller half, while the remaining 10 are the unpaired (smaller) *longifolia* chromosomes. The larger and smaller halves of the 10 bivalents separate and pass regularly to the poles of the heterotypic spindle, but the unpaired chromosomes are irregularly distributed or left out of the daughter nuclei. Later the pollen deteriorates. This result is strikingly different from that in the Oenothera hybrid, and, while perfectly in harmony with the idea of the pairing of threads in synapsis in Drosera, makes it highly probable, and in fact necessary, that the method of reduction in the Oenothera hybrid be different. This is a strong argument not only against pairing of maternal and paternal spirems in Oenothera, but in favor of the probability that reduction takes place in diverse ways in the two genera. A considerable amount of time has already been devoted to the study of reduction in this Oenothera hybrid, and an account will be published later. So far as observed it shows no differences in method from the account given here for the pure races.

The hypothesis of the pairing of parental spirems in synapsis in Oenothera being thus rejected, the other alternative remains, namely, that the double spirem results from a split; and this appears to satisfy all the facts. The observations have already shown that the spirem segments into a single chain of chromosomes. The description of events in Oenothera from synapsis on thus agrees in outline with the 1904 account of STRASBURGER (**30**) in Galtonia, and in general also with that of FARMER and MOORE (**9**) in Lilium, Osmunda, Psilotum, and Aneura, FARMER and SHOVE (**10**) in Tradescantia, and MOTTIER (**19**, **20**) in Lilium, Podophyllum, and Tradescantia. The belief of the writer is that some of these forms will be found to correspond more nearly with the account which involves a pairing of threads, and some with the account involving only a split.

Another important matter which requires mention at this time is the nature of the chromosome distribution which takes place on the heterotypic spindle in Oenothera. As already observed, the chromosomes even during spindle formation are frequently unpaired. This appears to be due to the weakness of the mutual attraction which ordinarily leads to pairing. Granting that homologous maternal and paternal chromosomes unite when pairing takes place, what are the possibilities regarding the unpaired chromosomes? Pairing insures ordinarily that the members of the pair will proceed to opposite poles of the spindle, and hence that the homologous maternal and paternal elements will enter different nuclei. There is no such certainty in the distribution of the unpaired chromosomes, so that it might be expected that in certain cases both members of a pair would enter the same daughter nucleus. It is important to note that this result is entirely independent of the origin of these chromosome pairs, whether from an end-to-end or side-by-side union of somatic chromosomes, or in any other manner, so that this question holds no necessary relation to the method of reduction. On the common cytological assumption that the chromosomes are qualitatively different (which has apparently been shown to be a fact in certain well-known cases in animals, that need not be cited), germ cells would occasionally arise lacking both members of a pair, and hence lacking the possibility of developing certain qualities. In this manner it is conceivable that a series of types might arise from the parent *O. Lamarckiana*, each lacking the possibility of developing a certain group of characters possessed by *O. Lamarckiana*.

On this view, which is suggested merely as a tentative hypothesis, we would have in the mutations of *O. Lamarckiana* an analytical process in which a series of types arises from the parent form, each lacking in a different group of qualities or capacities which the parent form possessed. This does not apply to *O. gigas*, however, which will be taken up at another time. The further bearings of this hypothesis on the mutation theory of DeVries will not be followed up in this discussion, but it may be pointed out here that such a hypothesis accounts for the absence of reversions of the mutants to *O. Lamarckiana*, and it may also account for some of the peculiarities of hybridization among the Oenothera mutants. I should therefore suggest that there may be a relation between the type of reduction in any organism and its variation and hybridization phenomena.

In Galtonia and probably also in Tradescantia there are apparently the same possibilities that both chromosomes of a pair may occasionally enter the same daughter nucleus. In other plant forms studied the attraction between chromosomes seems to be strong enough to keep the members of a pair together until their separation in the anaphase of the heterotypic mitosis. The segregation of the members of a pair into separate germ cells is thus insured. In cases where, as in Oenothera, the members of a pair do not always remain in contact, but are loosely arranged on the spindle, such a result as already suggested seems certain to occur in certain instances.

It has already been mentioned that occasionally one chromosome goes to the wrong pole of the heterotypic spindle. This is found to be the case particularly in the hybrids, for example, in the *O. Lamarcki-*

ana plants from the F_1 of *O. lata*×*O. Lamarckiana* (**13**), in which sometimes eight chromosomes pass to one pole and six to the other; but it may also occur rarely in the pure races. This matter was briefly discussed elsewhere (**14**). Assuming that the 14 chromosomes are in two similar sets of 7 each, and that homologous members of these sets conjugate except when there is a failure to pair, then when 8 chromosomes go to one pole and 6 to the other, both members of one of the pairs must have gone to the same pole. This probably takes place in cases where such members were unconjugated, for the purpose, or at any rate, the result of the pairing is in ordinary cases that one member of every pair shall be distributed to each pole. If, while two members of one pair thus go to one pole, the second member of another pair goes to the other pole, we should have an equal numerical distribution of chromosomes, but one daughter group would be lacking both members of one pair and the other would be lacking both members of another pair. It is highly probable that such a distribution occasionally takes place, though it would be less common than the case, already proved, where the members of a single pair are unilaterally distributed. It should be borne in mind that such cases are most likely to occur, not when the members of a pair are conjugated, but when they lie separately in diakinesis and on the spindle.

Miss LUTZ (**17**), from an examination of root tips, states that she has observed several individuals belonging to different strains having 15 chromosomes instead of 14. This is to be anticipated from the irregularities in chromosome distribution in reduction already mentioned. I have observed one such case in *O. lata*×*O. gigas* (**14**)—a certain plant having 20 chromosomes instead of 21. All the plants of *O. lata* (**12**) and *O. nanella* (**13**) thus far examined by me had 14 chromosomes, while Miss LUTZ (**17**) finds in root tips some *O. lata* plants with 14 and also some with 15 or she thinks possibly 16 chromosomes. She reports finding two *O. nanella* plants with 14 chromosomes and one with 15. Two *O. albida* seedlings are said to have 15 chromosomes and two *O. oblonga* plants 15, while a third has 14. Disregarding the possibility that these results might be due to the well-known variation in chromosome numbers in root tips, they are such as would be likely to arise in different individuals from the cytological irregularities I have already described. Whether there are external differences between the plants having 14 chromosomes and those of the same race having 15, is as yet unknown. But it is quite conceivable that no such differences will be found, for if the sporophyte chromosomes consist of two complete sets (and for a variety of reasons this seems the only tenable view at the present time if we assume qualitative differences at all), the presence of an additional chromosome, which is already present in duplicate, would scarcely be expected visibly to affect the plant.

ROSENBERG (26) has found an analogous situation in Hieracium. For example, *H. excellens* × *H. Pilosella* gives hybrids with different numbers of chromosomes. This he ascribes to the fact that the eggs of *H. excellens* differ in their numbers of chromosomes, which he finds is due to irregularities in chromosome distribution during the reduction divisions. The writer has pointed out elsewhere (**12**) certain similarities between the hybridization phenomena in Hieracium and Oenothera, and this seems to be a further similarity between the two genera.

ROSENBERG (27) has since shown that *H. excellens* produces three kinds of embryo sacs: (1) Normal embryo sacs which require fertilization for their development. These are presumably the only ones which can be hybridized. The egg cells in these sacs vary in their number of chromosomes owing to the fact that some of the chromosomes, lacking in "affinity," remain univalent (that is, fail to pair) during the heterotypic mitosis and are irregularly distributed. It is evident that this lack of affinity between chromosomes is similar to that in Oenothera. (2) In rare cases apogamous embryo sacs are formed after a single division of the megaspore mother cell, and without reduction. (3) More frequently the condition occurs which ROSENBERG calls apospory, in which tetrad formation takes place and then an adjacent cell of the nucellus enlarges, displaces the tetrad, and forms an embryo sac without reduction.

Summary

In conclusion a brief summary of the facts and considerations here presented will be useful.

1. In Oenothera the heterotypic mitosis is a reduction division, separating whole chromosomes which lie successively on the spirem. The homotypic mitosis is an equation division, separating the longi-

tudinal halves of the daughter chromosomes of the heterotypic mitosis. Whether an approximation of threads or a split in a single thread occurs in synapsis was not determined with certainty from the observations, but various considerations lead to the belief that in Oenothera the doubling is due to a split which closes up later, rather than to an approximation of separate spirems.

2. The conclusion that the method of reduction probably differs in different genera is based on two considerations: (1) the fact that in most of the recent accounts of synapsis and reduction in plants a side-by-side pairing of chromosomes from maternal and paternal spirems is described, while in Oenothera the members of a pair are arranged end to end on a single spirem; and (2) on differences in chromosome distribution during reduction in certain hybrids of Drosera and of Oenothera (see p. 25). If reduction took place in the same manner in both genera, the chromosome distribution during reduction in these hybrids with reference to the parental chromosome numbers should be the same in both, but this is not the case.

3. Pairing between the definitive chromosomes during diakinesis and the prophase of the heterotypic mitosis does not always take place, owing to a weak attraction between the chromosomes. This allows irregularities of distribution in the heterotypic mitosis, so that both (unpaired) chromosomes belonging to one pair will *occasionally* enter the same daughter nucleus (see p. 26). Germ cells will thus arise, from which both members of a given pair of chromosomes are absent.

4. If we assume qualitative differences between the chromosomes or parts of them, various types would be expected to originate in this manner, each of them lacking the ability to develop certain qualities possessed by the parent form. On this view the mutations of *Oenothera Lamarckiana* are an instance of a process of analysis by which from the parent form arises a series of types, each lacking in certain characters or capacities possessed by the parent. This hypothesis would account for the absence of reversions among Oenothera mutants, and perhaps also for some of the peculiarities of hybridization in Oenothera. This matter will be considered at another time. This explanation does not apply to all the mutants, however; for example, *O. gigas*.

5. It is suggested that there is probably a direct relation between the events of reduction in a given genus and its variation, as well as its hybridization phenomena.

I desire to express my thanks to Professors JOHN M. COULTER and CHARLES R. BARNES for valuable suggestions and adequate facilities in connection with this work.

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EXPLANATION OF PLATES I–III

The figures were drawn with the aid of a Bausch & Lomb camera lucida. All except *figs. 1* and *19* were drawn under a Zeiss apochromatic objective 2^{mm} ap. 1.30, with a Zeiss compensating ocular 18. The figures are reduced one-fourth in reproduction, giving a magnification of nearly 3000 diameters. *Fig. 1* was drawn under a 2^{mm} objective and compensating ocular 6; *fig. 19* under B. & L. objective $\frac{1}{16}$ N. A. 1.32 and Zeiss ocular 18.

PLATE I

FIGS. 1, 2.—Young meristematic cells of anther primordium showing one large nucleolus and several smaller ones, and chromatic masses adherent to the nuclear membrane.

FIG. 3.—Longitudinal section of anther, showing size relations of nucleoli in sporogenous, tapetal, and wall cells.

FIG. 4.—One sporogenous cell from stage of *fig. 3*, previous to synapsis; cytoplasm somewhat vacuolate.

FIGS. 5, 7–9.—Nuclei at same stage, showing fusions of nucleoli.

FIG. 6.—Two nucleoli of equal size; an unusual condition.

FIG. 10.—Several small nucleoli, and no indication of fusion.

FIG. 11.—Nucleoli of young pollen grain nucleus.

FIG. 12.—Beginning of synaptic contraction; the reticulum has contracted from the nuclear membrane on all sides, leaving several loops attached to the membrane; on the side on which the reticulum retains the curved outline of the nuclear membrane the latter has been drawn inward attached to the threads; on the rest of the circumference, between the loops, the nuclear membrane remains *in situ;* the cytoplasm is perfectly fixed.

FIG. 13.—Another contraction stage, showing loops attached to the nuclear membrane, which is intact.

FIG. 14.—A slightly later stage of contraction, in which the rearrangement of threads is taking place.

FIG. 15.—Synapsis; dark-staining bodies are still held in the meshes of the spirem; a small nucleolus, usually about the size of a chromosome, is generally present in addition to the large nucleolus.

FIG. 16.—After synapsis; the thread thicker and shorter and loosely coiled.

FIG. 17.—Slightly later stage than *fig. 16*, and less deeply stained; thread shows the characteristic light and dark areas; indications of parallel threads in two places; edge of thread may be even or moniliform.—5 μ.

FIG. 18.—Later stage; thread much shortened and greatly thickened and entering upon second contraction phase; nucleus uncut.—10 μ.

FIG. 19.—Higher magnification of a portion of the thread in *figs. 20* and *21*.

PLATE II

FIG. 20.—Second contraction stage; a pair of chromosomes cut off from spirem; nucleus uncut.—10 μ.

FIG. 21.—Second contraction stage; nucleus uncut.

FIG. 22.—Uncoiling from second contraction stage; pair of chromosomes detached; nucleus uncut.

FIG. 23.—Spirem segmented in three places, each segment showing constrictions which will form the chromosomes; certain chromosomes already detached; nucleus uncut.

FIG. 24.—Constriction of spirem has proceeded farther, the chromosomes being elongated bodies with irregular margins like the spirem, and connected by rather

thick "linin" bands; pair of chromosomes detached earlier lies at side of nucleus; *n*, small nucleolus; nucleus cut.

FIG. 25.—Spirem more or less completely segmented into chromosomes while still in the second contraction stage; preparation considerably destained; 13 chromosomes in view.

FIG. 26.—Spirem segmented, showing chain of eight chromosomes and three pairs; nucleus uncut.

FIG. 27.—Chain of six chromosomes, and probably four pairs; linin connections between members of a pair not always visible; nucleus uncut.

FIG. 28.—Fourteen chromosomes; several small nucleoli; nucleus uncut.

FIG. 29.—Fourteen chromosomes, including five pairs more or less closely associated; linin connections not visible; one pair of chromosomes has already contracted into the globular shape.

FIG. 30.—Fourteen chromosomes, several in pairs; apparent inequalities in size due to positions in which some of the chromosomes are lying.

FIG. 31.—Slightly later stage; the fourteen chromosomes contracted into the globular or pear-shaped definitive form; linin connections longer and extremely delicate; nucleus uncut.

FIGS. 32–34.—Other groups in diakinesis, showing various peculiarities of chromosomes.

FIG. 35.—Peculiar case of spindle formation; three nucleoli present and fourteen chromosomes, including three or four pairs.

FIG. 36.—Multipolar stage of heterotypic spindle; two more or less closely united pairs of chromosomes present.

PLATE III

FIG. 37.—Same as *fig. 36;* an unusual case in which all the chromosomes are closely joined in pairs; seven such pairs present and a small nucleolus.

FIG. 38.—Heterotypic spindle in metaphase; spindle has usually more mantle fibers than in *O. Lamarckiana;* chromosomes usually loosely arranged in equatorial region of spindle.

FIG. 39.—Late anaphase; an uncommon case; daughter chromosomes have failed to divide, and fibrillae are scattered in cytoplasm at side of cell; chromatic staining material also present.

FIG. 40.—Telophase of heterotypic mitosis; exceptional case, in which a rather sharp pointed spindle is formed at side of cell; it probably originated from the fibrillae shown in *fig. 39*.

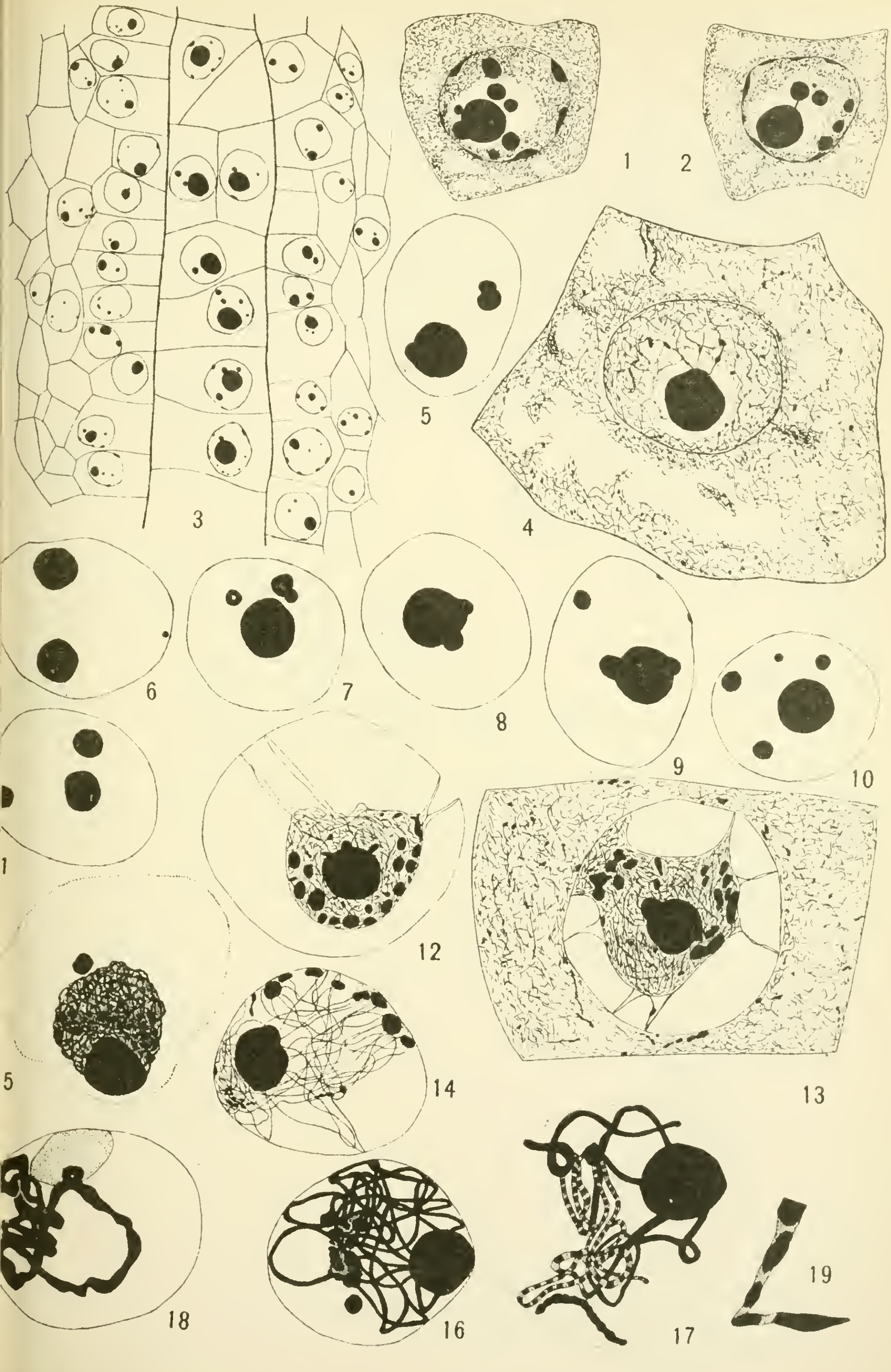
FIG. 41.—Early anaphase of homotypic mitosis; small nucleolus having the characteristic appearance, present on one of the spindles.

FIG. 42.—The single case of extra nuclei observed in *O. rubrinervis* pollen mother cells.

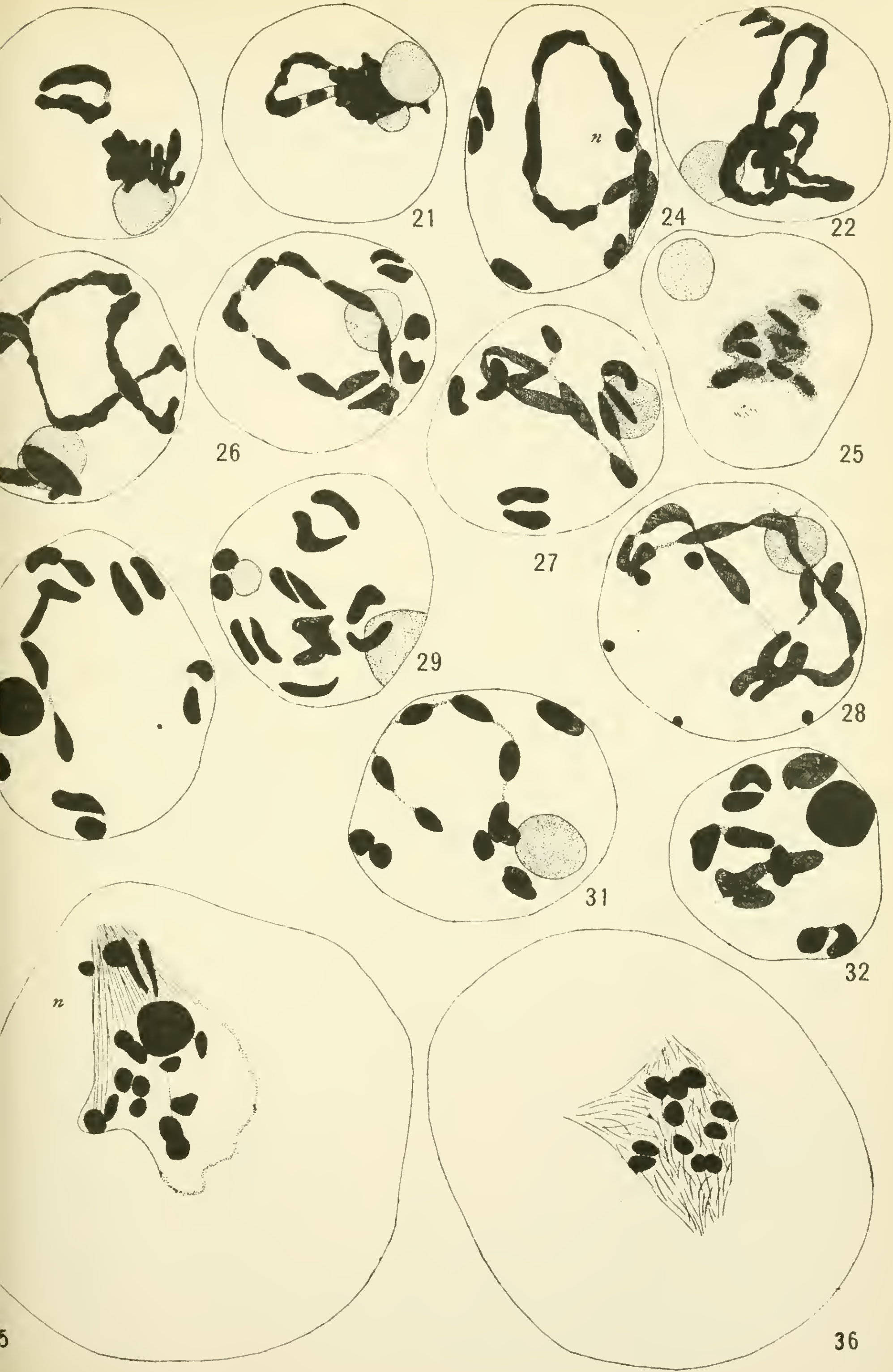
FIGS. 43, 44.—Nuclei from telophase of second mitosis, passing into resting condition.

FIGS. 45, 46.—Protoplasmic connections between mother cells.

PLATE I



GATES on REDUCTION in OENOTHERA



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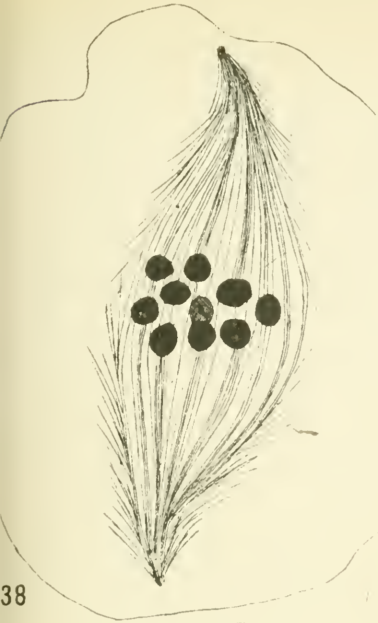
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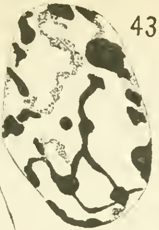
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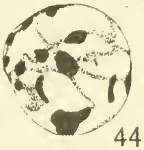
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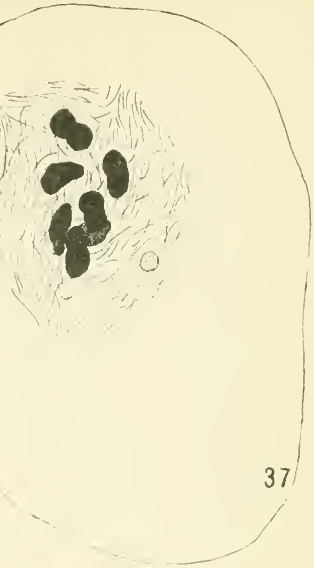
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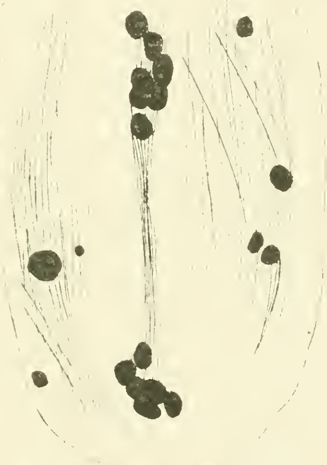
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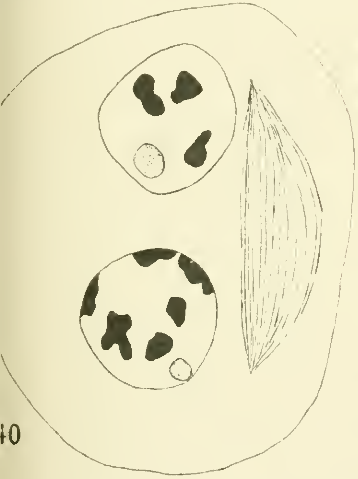
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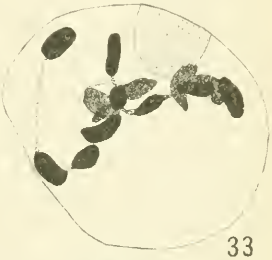
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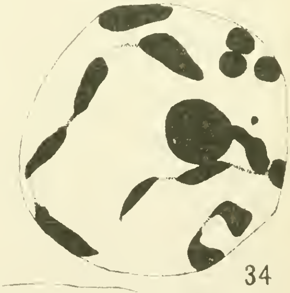
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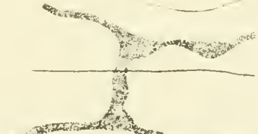
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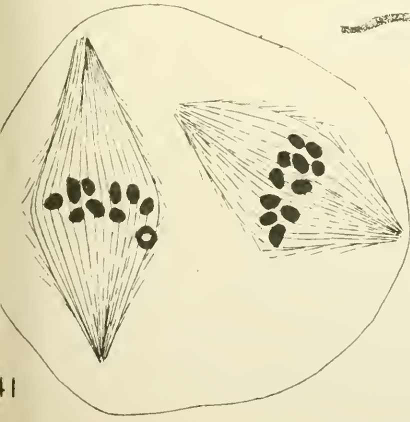
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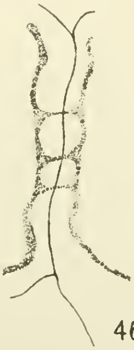
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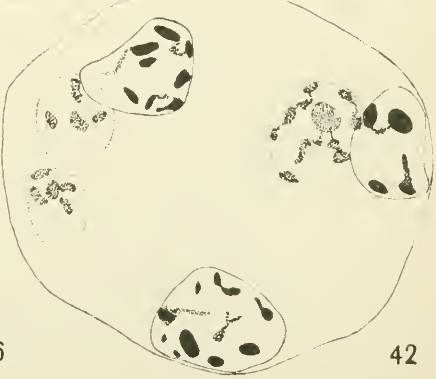
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GATES on REDUCTION in OENOTHERA

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THE STAMINATE CONE AND MALE GAMETOPHYTE OF PODOCARPUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 114

(WITH NINE FIGURES AND PLATES VIII AND IX)

L. LANCELOT BURLINGAME

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THE STAMINATE CONE AND MALE GAMETOPHYTE OF PODOCARPUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **114**

L. LANCELOT BURLINGAME

(WITH NINE FIGURES AND PLATES VIII AND IX)

Knowledge of the gametophytes of the Podocarpineae is at the present time limited to that contained in three brief papers. In 1902 COKER (**3**) published an account of two species of Podocarpus; in June 1907 JEFFREY and CHRYSLER (**5**) added some new facts concerning Podocarpus and Dacrydium; and in September of the same year Miss YOUNG (**14**) gave an excellent résumé of the subject of the male gametophyte and added several new and interestesting details for Dacrydium. COKER records that in *Podocarpus coriacea* there are produced two primary prothallial cells, both of which may divide amitotically; a tube nucleus; and a primary spermatogenous cell, which gives rise to a stalk cell and a body cell. The body cell undergoes nuclear division and one of the nuclei is then extruded from the cytoplasm, leaving a single functional male cell. JEFFREY and CHRYSLER add to this the interesting details that from the two primary prothallial cells there may arise by subsequent division as many as eight prothallial cells. Of still greater interest is the record of the "proliferation" of the primary spermatogenous cell, whereby there arise three cells placed transversely, the middle one of which is considerably larger than either of the others. Miss YOUNG reports that in Dacrydium one or both of the primary prothallial cells may divide once. From the primary spermatogenous cell arise two cells, one of which is usually sterile, the other of which functions as the body cell. The interesting observation is made that there is a tendency for both derivatives of the primary spermatogenous cell to function as body cells.

The material for the present study was secured through the kindness of Dr. L. COCKAYNE of Christchurch, New Zealand, and Miss INEZ FRANCES STEBBINS of the Huguenot College at Wellington, Cape Colony, South Africa. To both are due our thanks for their kindness in obtaining the material at no inconsiderable personal inconvenience. Though the collections were necessarily few, yet they include a considerable range of developmental stages in each of the species. The material was fixed in a 5 per cent. solution of commercial formalin in 70 per cent. alcohol. The fixation has proved unexpectedly good, except for stages from the rounding-up of the mother cells to the time when the microspores have a good firm wall and abundant contents. The shrinkage and general distortion within the limits just mentioned have rendered necessary the omission of a number of what appear to be rather interesting and perhaps important details. Some of these will be mentioned briefly in the general account. It is hoped that other material so killed and fixed as to show details of cytological structure will be in hand within the near future. The preparations have been imbedded in 54° paraffin, cut 3–5 μ, and stained in Haidenhain's iron alum hematoxylin stain, alone or counterstained with various aqueous stains such as orange G, Bismarck brown, erythrosin, etc., and with the saffranin gentian-violet combination. Three species have been examined, though not covering exactly the same ground.

Observations

The cones of *P. totarra Hallii* are of a generally oval shape, varying from 3 or 4 to 15 or 20^{mm} in length, and from 2 to 4^{mm} in diameter. They are usually in pairs, one being large and one small. The base of the cones is surrounded by several closely appressed, rigid, scalelike bracts. The peduncle is quite short or almost lacking. The cones of *P. nivalis* are somewhat shorter, not paired, and decidedly slenderer. The cones of the third species (the label of which was lost in transit) are similar in shape to the first ones, but are smaller. In other respects the cones are very similar. *P. totarra* is one of the tallest of forest trees and *P. nivalis* a very small shrub, sometimes creeping. The third species may be *P. elongata*, a common forest tree of the South African region. This is a mere inference from its abundance in the region from which this material

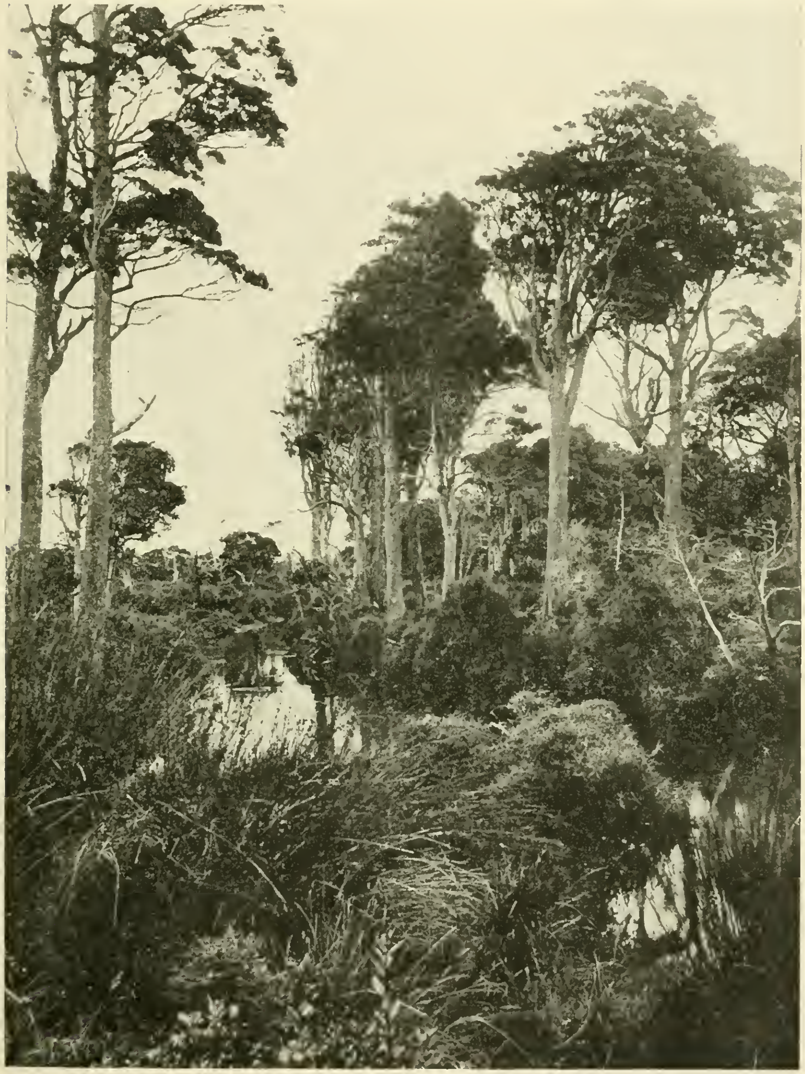

FIG. A.—Group of *P. dacrydioides* in remnant of taxad forest, N. Z.—Photograph by J. CROSBY SMITH.

was received, and from the fact that I have received other material from the same source belonging to this species.[1]

In cross-section a staminate cone shows about ten to twelve sporophylls, each with two sporangial cavities (*fig. 1*). The vascular system seems to be rather weakly developed, but shows a distinct endarch collateral bundle (*fig. 3*) corresponding to each sporophyll. Just outside of the bundle there usually is found a resin canal (*fig. 1*). The resin canals contain little material of any sort in the preparations available for study. The distinctly glandular cells lining them (*fig. 2*) suggest, however, that they are functional. The number varies in different strobili, but in those examined did not fall below six nor exceed eighteen or twenty.

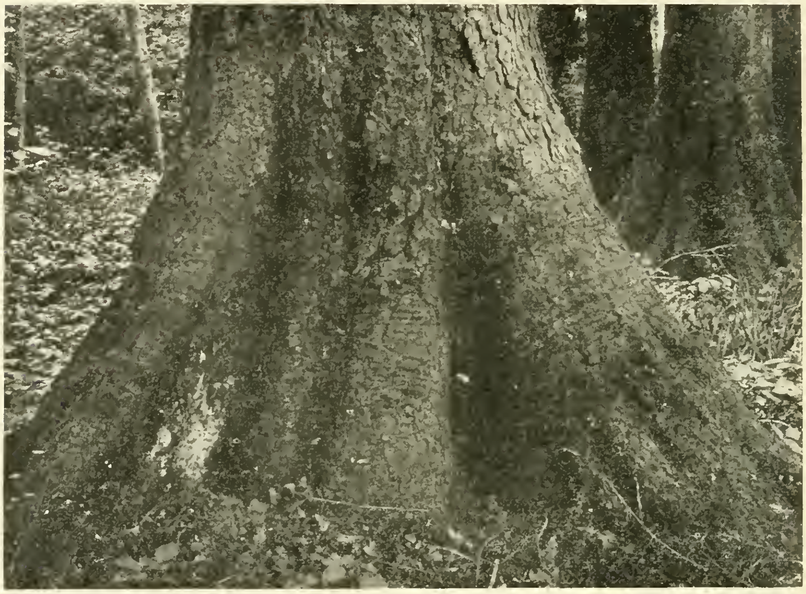

FIG. B.—Buttressed base of *P. dacrydioides*, in ancient forest of Canterbury Plain, N. Z.—Photograph by L. COCKAYNE.

The sporophylls as seen from the upper surface are somewhat spatulate, with a rather pointed apex. *Fig. 4* presents an outline of a longitudinal section somewhat to one side of the middle, and will give an idea of the side view, as well as the relation of the sporangial cavity to the sterile portion of the leaf. *Fig. 6* shows a cross-section of a sporophyll near the middle of the sporangium. The sporangia are somewhat less prominently exposed on the under side than those of Pinus, for example. A single weakly defined vascular bundle (*fig. 6*) traverses the upper part of the central sterile septum and is sometimes accompanied by a resin canal lying below it and between the sporangia. The wall of the sporangium varies in thickness somewhat, but in general on the freely exposed part it is about four or five cells thick (*fig. 5*). One or two of the inner layers are slightly differentiated as a tapetum. This is not very evident and does not occur until the sporogenous tissue has nearly reached the mother cell stage. The tapetum does not long persist, but disappears as a functional tissue about the time the young spores have formed a thick wall and wings. All of the wall proper, except the outermost layer of thickened cells, may break down before the spores are shed.

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[1] We have received from Dr. COCKAYNE two excellent photographs of *P. dacrydioides*, which are reproduced on account of their general interest (*figs. A* and *B*).

The earliest stages of the sporogenous tissue observed (in *P.* sp.) lacked one or two divisions of the mother cell stage. *Fig. 7* will serve as a typical representative cell of the sporogenous tissue at this stage. The walls are thin and delicate, as is usually the case, and the cytoplasm stains somewhat more densely than that of the wall cells. It was possible to secure very good preparations of this, considering the nature of the killing and fixing agent. The cells were slightly shrunken, so as to have pulled away in many places from one another or from their own walls. *Fig. 7* will show the principal facts. The cytoplasm does not seem to present either a typically reticulated or a foam structure, but rather resembles a sort of flocculent precipitate scattered more or less irregularly through the cell. These flocculent masses may assume a sort of feathery, filamentous, more or less branched form, or they may appear as scattered masses of irregular shape. The filaments and other masses do not seem to be connected in any definite manner, but to be distributed through the cell by chance. No stored food can be detected microscopically in the cytoplasm at this time, though the parenchymatous cells of the axial portion of the strobilus contain considerable quantities of large starch grains. The wall cells of the sporangium do not at this time, as in some sporangia (Ophioglossum, for example), contain starch, but are relatively poor in cytoplasm and cytoplasmic inclusions. There is relatively little change in the cytoplasm of the sporogenous tissue as growth advances, except that it grows gradually less dense until at the time of the formation of the young spores there is almost no stainable substance left.

The nuclei of the sporogenous cells (*fig. 7*) previous to the mother cell stage show a structure that is very like that of the cytoplasm just described, except that it is denser and there is more of a tendency to aggregate itself into disconnected masses. There are one or more large nucleoli present in the nucleus at this stage, as there seem to be in the resting nuclei of all the stages of all the tissues examined.

As the nucleus approaches division, the nuclear substance begins gradually to arrange itself into filaments or strands (*fig. 8*) which may end freely or anastomose with other filaments or masses. About the same time the hitherto nearly uniformly staining material begins to stain differentially, so that filaments seem to link together knots of more deeply staining material. Whether this indicates a difference of substance, as some think, or merely a difference in the state of aggregation, as has been recently maintained by several authors, cannot be said with certainty. However, the gradual derivation of this structure from the preceding one, in which everything seems homogeneous, would certainly point to the latter interpretation as the correct one. During this time the nucleolus (or nucleoli) gradually loses its density, as shown by the staining reactions. It continues to do so until it finally does not stain at all or has disappeared entirely. *Fig. 9* shows a surface view of a nucleus in which the filaments have become more regular and somewhat thicker. In *fig. 10* is shown a spirem, into which the filamentous stage gradually passes, in which the deeply staining knots on the spirem are unusually prominent. The figure shows nearly all of the spirem that was included in a 3-μ section; therefore it probably contains one-third to one-half of the entire spirem, since the nucleus is about $7 \times 10\,\mu$. In some cases the spirem consists of darkly stained masses connected by much smaller threads, and in others, as shown in *fig. 10*, by broader strands which seem to be of different material because of the considerable difference in depth of staining.

The spirem gradually becomes denser and ramifies all through the nucleus (*fig. 11*), the nucleolus disappears, and finally the spirem breaks into elongated loops, which arrange themselves into a tangled mass at the equatorial plate. The exact method of division was not made out owing to the close tangling of the long chromosomes. In the anaphase the chromosomes appear as twenty-four straight elongated rods of about half the length of those entering the mitosis (*fig. 12*).

As the chromosomes approach the poles, the polar ends draw together and the others spread out, so that the daughter nucleus usually has a concavity on the polar side. The chromosomes gradually lose their density and put out processes on one side or the other to fuse with those put out by neighboring ones, thus establishing a sort of reticulate structure (*fig. 13*). This gradually passes back into the stage shown in *fig. 7*. In the reorganizing nucleus one large nucleolus may appear or several small ones, but I did not attempt to discover the origin of them or their relation to one another.

When the cells have reached the mother cell stage, they break loose from one another and round up in the usual manner. As already remarked, the mother cells killed and fixed very poorly, so that only a very meager and perhaps uncertain account of them can be given. About the time the mother cells round up many of them begin to abort; this abortion seemingly may occur as late as the young spores. Up to this time and on to the time of the divisions in the young microspores the development of the sporogenous tissue seems to be nearly simultaneous.

After the mother cells have rounded up, the nucleus soon passes into a condition in which its contents stain so nearly uniformly that it has not proved possible to disentangle its separate constituents. If stained deeply, it appears absolutely uniform, and if the stain be then slowly and gradually drawn, the homogeneous appearance gives way to a sort of pebbled one. Sometimes it looks as if this might be due to the parts of a large and long spirem being crowded closely together. This interpretation is strengthened by the fact that in some cases one can see what looks like the end of a loop pushing out the nuclear membrane. In other cases the appearance is more that of a great many lumps of some plastic substance thrown into a tight mass. Whether these appearances are normal or not I cannot say. That they represent the only appearances that the present material shows is certain. In the face of the numerous and fairly uniform accounts of sporogenesis in which no such phenomena have been recorded, it is certainly entirely proper at present to ascribe these unusual phenomena to the effects of the reagents. Nothing any more nearly resembling synapsis was seen. It should be noted that of some hundred strobili cut only five showed cells in this stage. However, three of these strobili are the only ones that have shown the reduction divisions. A few sporangia had cells like those shown in *fig. 14*, where the chromosomes are beginning to appear, being almost completely concealed in a mass of granular matter. In the figure the difference in intensity of stain between the chromosomes and the imbedding substance has been greatly exaggerated for the sake of clearness. One gets two views of these developing chromosomes, one in which the chromosomes are mostly seen in cross-section and one in which they lie lengthwise. In some fashion (which the density and abundance of the imbedding substance prevent being made out clearly) out of this comes a rather sharply pointed spindle at whose equator are arranged the short apparently bivalent chromosomes (*fig. 15*). Some preparations seemed to favor the idea that these apparently bivalent chromosomes then split longitudinally. After passing to the poles (*fig. 16*) they became surrounded by a nuclear membrane, but did not, in the few preparations of this stage observed, lose their identity in a resting nucleus. A very few cases of the homotypic mitosis are shown in the preparations and all of these are in the telophase, as shown in *fig. 17*. Walls are not formed till after second division.

Beyond the telophase of the second division the preparations show nothing until after the formation of the wall around the young spores. These are so badly shrunken and distorted that no attempt has been made to figure them. It is observable, however, that the nucleus is at first rather small and oval and lies to one side of the spore cavity, most of which is occupied by an enormous vacuole. *Fig. 18* shows a microspore after the contents have become more abundant. At this time starch may or may not be present; later it becomes abundant and occurs in relatively large grains (*figs. 30, 31*). The wall has a thick exine much roughened on the outer surface and a distinct intine. The spore coat is usually much thinner on the side away from the point where the prothallial cells will later lie. The external appearance of the pollen grain bears a considerable resemblance to that of Pinus, except that it is smaller, rarely reaching more than 35 μ in the greatest diameter (exclusive of the wings).

The microspore nucleus is large (*fig. 18*) and contains apparently two sorts of material. There is a more deeply staining substance distributed in irregular masses through a very fine ground substance. As the nucleus prepares for division, the amount and density of the more deeply staining substance increases in amount, assumes a more definitely reticulated structure, and finally passes into a rather thick and comparatively short spirem (*fig. 29*). This spirem breaks up into the chromosomes (*fig. 28*), which often still show the distinct segmentation into lighter and darker segments that has already been referred to in discussing the sporogenous tissue. This segmentation is not always found in the chromosomes or even in the spirem. It might be supposed that the different appearance is due to differences in the depth of stain, but this does not seem to be true. The chromosomes are rather long and considerably twisted at the metaphase, but in dividing and passing to the poles they apparently shorten up (*fig. 31*). What has been said of the first division applies equally well to the next, so far as the few mitoses observed show.

One or two prothallial cells are cut off and then the antheridial initial divides to produce the tube nucleus and the primary spermatogenous cell (*fig. 32*). In *P. nivalis* two primary prothallial cells, which do not subsequently divide, seem to be the rule, though one example of the division of the first prothallial was observed. The pollen in the same sporangium varies in the stage of development from microspores to the stage shown in *fig. 32*. These sporangia were, in some cases at least, shedding their spores.

In *P. totarra Hallii* one primary prothallial cell may be cut off, after which the free nucleus divides to form the primary spermatogenous cell and the tube nucleus (*fig. 21*); or two primary prothallial cells may be cut off before the tube nucleus is separated from the primary spermatogenous cell (*figs. 20, 22*). When only a single primary prothallial cell is cut off, it may (*fig. 19*) or may not (*fig. 21*) divide again. Far more frequently it does divide anticlinally. No case in either species has been observed where either prothallial cell divides periclinally. In case two prothallial cells are cut off, both usually divide anticlinally (*figs. 23, 26, 27*), although the division may be long delayed or fail wholly (*text fig. 4*). The first one may remain undivided, or in rare cases may divide twice, so that the first tier of prothallial cells contains four cells. The second one almost always divides once and usually twice. Thus there may be one, three, four, six, or eight prothallial cells; six is much the commonest number, and one or eight the rarest. In case only one prothallial cell is cut off, its nucleus

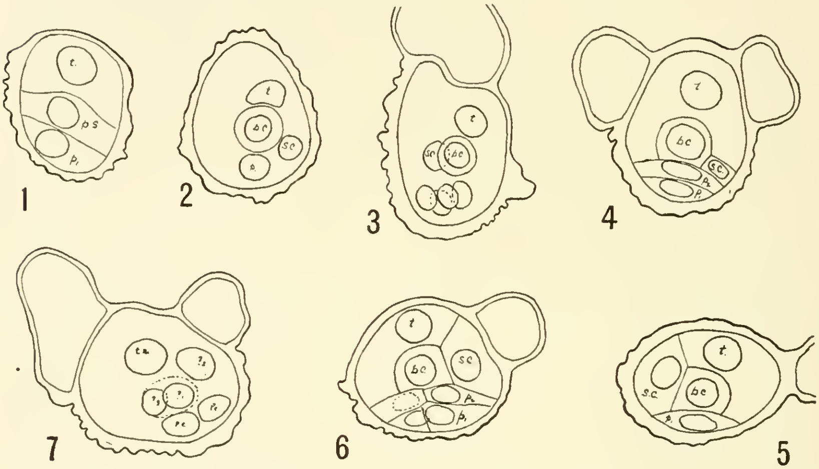

FIG. 1. Transverse section of a male gametophyte consisting of one prothallial cell (p_1), the primary spermatogenous cell (*ps*), and the tube nucleus (*t*).—FIG. 2. Male gametophyte in which the primary spermatogenous cell has divided into the body cell (*bc*) and the stalk cell (*sc*), and in which all the gametophytic walls have broken down.—FIG. 3. Same as the preceding except that there are four prothallial nuclei instead of one; it is uncertain whether they have been derived from one or two primary prothallial cells.—FIG. 4. A gametophyte with two primary prothallial cells (p_1, p_2) which have not divided, though the pollen is presumably ready for shedding.—FIG. 5. A gametophyte with a large stalk cell placed transversely to the axis of the body cell.—FIG. 6. Same as preceding except that there are four derivatives of the primary prothallial cell.—FIG. 7. A gametophyte in which the origin of the free nuclei is not easily traced; see text for discussion.

remains large and the cell itself is often much larger than when two are cut off (*text fig. 1*). Sometimes the three resulting nuclei then lie in a row across the spore (*text fig. 1*) and differ but little from one another in appearance. The walls separating them then run nearly straight across, and it is only by courtesy that the tube nucleus can be said to be free in the spore cytoplasm. To all intents and purposes it is as much walled-off into a cell of its own as the prothallial nucleus, for both eventually are freed in a common cytoplasm (*text fig. 2*). Further, it is to be noted that in this case the prothallial cell does not have a fixed position with respect to the wings, as in the ordinary course of events.

After the tube nucleus has been cut off, the primary spermatogenous cell divides transversely, giving rise to two cells (*fig. 25*). Usually these cells differ markedly in size, the smaller lying to one side and the larger lying nearly in the center of the pollen grain (*fig. 26*). In this case one may safely use the usual terms for them and speak of the larger as the body cell and the smaller as the stalk cell. It not infrequently happens that the two derivatives of the generative cell are about equal in size (*figs. 24, 27; text figs. 5, 6*), though even then one is centrally placed and the other laterally. Before the division of the generative cell, the second primary prothallial cell has usually divided at least once. In this case the generative cell often sinks down among its derivatives (*figs. 23, 27*). The stalk cell is sometimes placed transversely to the body cell (*text figs. 5, 6*). Whether these large stalk cells will produce male cells or not can only be conjectured, though the fact that some of them seem to retain their cytoplasmic envelope, almost as distinct from the cytoplasm as the body cell itself, up to a certain early stage, would lead one to suppose that in some cases they might do so. *Text fig. 7* may show three derivatives of the generative cell and two prothallial cells in addition to the tube nucleus. One of the three derivatives of the generative cell has no cytoplasmic sheath and is probably a stalk nucleus; the two lying close together are ensheathed, though I have been unable to ascertain definitely whether each has a distinct sheath or whether both lie in a common sheath, or whether perhaps one of them is not merely beneath the cytoplasmic sheath of the other. If either the first or second supposition is true, there still remains the question whether the two cells are body cells or male cells. If the third supposition is true then there are four prothallial nuclei, a generative cell, and the tube nucleus.

In a number of preparations there seemed to be a cell on the side of the body cell opposite the stalk cell (*fig. 27*), but in no case could I satisfy myself of the presence of a nucleus; though in view of the figures published by JEFFREY and CHRYSLER it was diligently looked for. In *fig. 27* is shown a mass at one end of this "cell," which may stand for a degenerating nucleus, although I think not. It is very indistinct and may very well be a mere aggregation of slightly denser cytoplasm. By constructing a model of the cell complex, where the body cell has sunk down between the cells of the second tier of the prothallus, and then cutting it so that the section will pass about centrally through stalk and body cell and include most of one-half of them and take a slice off the prothallial cells lying beyond them, it is possible to get preparations which show cell walls in the position shown in the figure. Hence it is not certain that in this species there are two lateral derivatives of the generative cell, as seems to be the case in the species mentioned by JEFFREY and CHRYSLER (**4**).

The dividing nuclei of the species of Podocarpus here investigated show uniformly twelve chromosomes in cases where they could be certainly counted (*figs. 14, 27, 32*), and twenty-four were counted several times, with less certainty, in the sporogenous tissue of *P.* sp. In this it conforms to the count for all other gymnosperms so far as known except Taxus, with eight and sixteen (STRASBURGER **12**), and Sequoia, with sixteen and thirty-two (LAWSON **7**).

It is not possible to speak with certainty concerning the stage at which the pollen is shed without knowing over how long a time shedding continues, and without actually having gathered pollen as it is shed naturally. But from cones that were apparently ready to shed their pollen, stages were obtained in *P. nivalis* running from the microspore to gametophytes with two prothallial cells, generative cell, and tube nucleus. It often happens that both extremes may be found in the same sporangium. In *P.* sp. no cones were apparently old enough to shed their pollen, though the oldest had essentially the same structure as those of the species just mentioned. In the cones of *P. totarra Hallii* the range appears to be still wider, for the oldest stages shown in the plates (*figs. 26, 27; text fig. 7*) occurred along with the youngest. In some cases the cell walls were still intact, while in other pollen grains not more advanced, the nuclei and body cell were free in the common cytoplasm (*text figs. 1, 3, 7*).

Discussion

The facts presented in the preceding account are in the main confirmatory of those already reported by Coker and by Jeffrey and Chrysler for other species of Podocarpus; hence their main value is that they offer additional evidence that the phenomena described by these authors are not limited to the species studied by them. These authors and others as well have called attention to the resemblances to the Abietineae in the winged pollen, thick sporangium wall, and general similarity of the staminate strobilus.

The main interest centers about the development of the male gametophyte. Coker (**2**) reported two primary prothallial cells in *P. coriacea*, which divide amitotically. Jeffrey and Chrysler (**5**) have recorded as many as eight in the species studied by them. Miss Young (**14**) has also recorded as many as four in species of Dacrydium. Thompson (**13**) has found 30 to 40 prothallial cells in Agathis; and Lopriore (**8**) has reported a cell complex in Araucaria that reaches about 15 in number before the walls break down, when the freed nuclei may then further divide until as many as 44 may be found in the pollen tube. He interprets these as spermatogenous cells and cites the case of Cupressus as analogous. Chamberlain (**2**) has already called attention to the fact that the drawings seem to indicate that these nuclei are really prothallial and that there is only one body cell. In this view Miss Young (**14**) has concurred, as it would seem most students of gymnosperms must. Renault (**10, 11**) and Oliver (**9**) have described the multicellular pollen grains of the Cordaitales and Stephanospermum, where as many as 20 cells are found in the male gametophyte; whether they are prothallial or spermatogenous is uncertain.

Caldwell (**1**) has reported as many as nine or ten body cells in Microcycas, along with a single stalk and single prothallial cell. Chamberlain in a forthcoming paper reports that Ceratozamia occasionally has four sperms. Juel (**6**) found a variable number of sperm cells (it is not clear whether he means body cells or male cells) in *Cupressus goweniana*.

It thus seems to be an established fact that more than two prothallial cells and more than two sperms occur in widely unrelated gymnosperms. Though we may all be willing, perhaps, to accept this statement of facts, there is no such unanimity of opinion as to the interpretation of them. It is obvious that one may adopt either of two views: (1) One or two prothallial cells were found in the primitive gymnosperms and in some cases these have divided to form a complex. This would be JEFFREY'S (**5**) "coenogenetic proliferation." In support of this view he has urged that a multiplication of prothallial cells is correlated with "protosiphonogamic" fertilization in the Araucarineae, "since the length of the pollen tube, in the absence of any special conductive tissue, such as is found in angiosperms, calls for a greater development of prothallial tissue." This line of reasoning could hardly be extended to Podocarpus or to any other case now known. If one could find some physical cause applicable to all cases, he might believe that this multiplication of cells is a thing of recent origin in each case. Furthermore, the idea that a multiplication of cells would be forthcoming just when needed is more teleological than accords with modern physiological teaching. If we do not accept this explanation, as I think we cannot, we may turn to the other possibility. (2) Primitive gymnosperms had a multicellular prothallial tissue, and not unlikely a spermatogenous complex of several cells. The evidence for this view is of two sorts, historical and theoretical. Historically we know that the pollen grains of certain Cordaitales (**10**) and Cycadofilicales (**9, 10, 11**) had a considerable tissue of some sort. Whether it was spermatogenous or prothallial is not very material, since this view assumes that both sorts existed somewhere in the ancestry of living gymnosperms and that by a gradual reduction each sort has been reduced to one or two cells. It is of interest to note that all the heterosporous pteridophytes show the same tendency, each of the living genera having a single prothallial cell and in some cases producing as few as four sperms. This assumption makes it easy to account for the facts already related. We have but to suppose that in these cases the forms showing these peculiarities have merely retained their primitive characters or, in cases where they occur only occasionally, that it is a reversion to ancestral conditions. JUEL (6) has already suggested this explanation for the case of Cupressus, and has even gone so far as to suggest a possible sequence in the reduction. At this point one must be on guard against supposing that types which illustrate this reduction series necessarily, or even probably, stand in the same series phylogenetically. It seems to the writer that if this fundamental principle be firmly grasped, it is entirely unnecessary to suppose that because Podocarpus and Pinus are clearly related and because Pinus is older historically, therefore the multiplication of prothallial cells in Podocarpus must be coenogenetic. One need but assume that both are derived from a common ancestral stock and that one has retained the prothallial complex and the other has lost it. Of course if one believes that Podocarpineae have come directly out of living Abietineae, this explanation would not hold. While it is true that the latter are known as far back as the Carboniferous (4) and the former are not known to be nearly so old, it is equally undeniable that we know but little of the plant remains of those parts of the world in which their remains would be most likely to be found.

Summary

1. There are in the species of Podocarpus studied two prothallial cells which may or may not divide. There may be as many as eight prothallial cells in two tiers derived from the two primary ones.
2. Division in the prothallial tissue is mitotic and the prothallial cells do not degenerate.
3. There is a stalk cell and a body cell, sometimes differing very little from one another in appearance; whether both may produce male cells is yet uncertain.
4. The number of chromosomes is twelve and twenty-four.
5. There may be a variable number of cells or free nuclei in the pollen grain at the time it is shed.

Acknowledgments are due to Professors JOHN M. COULTER and CHARLES J. CHAMBERLAIN of this laboratory for valuable advice and criticism during the progress of this investigation.

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EXPLANATION OF PLATES VIII AND IX

The drawings were made with a Bausch and Lomb camera lucida and Zeiss apochromatic lenses and compensating oculars. The plates have been reduced one-half in reproduction.

PLATE VIII

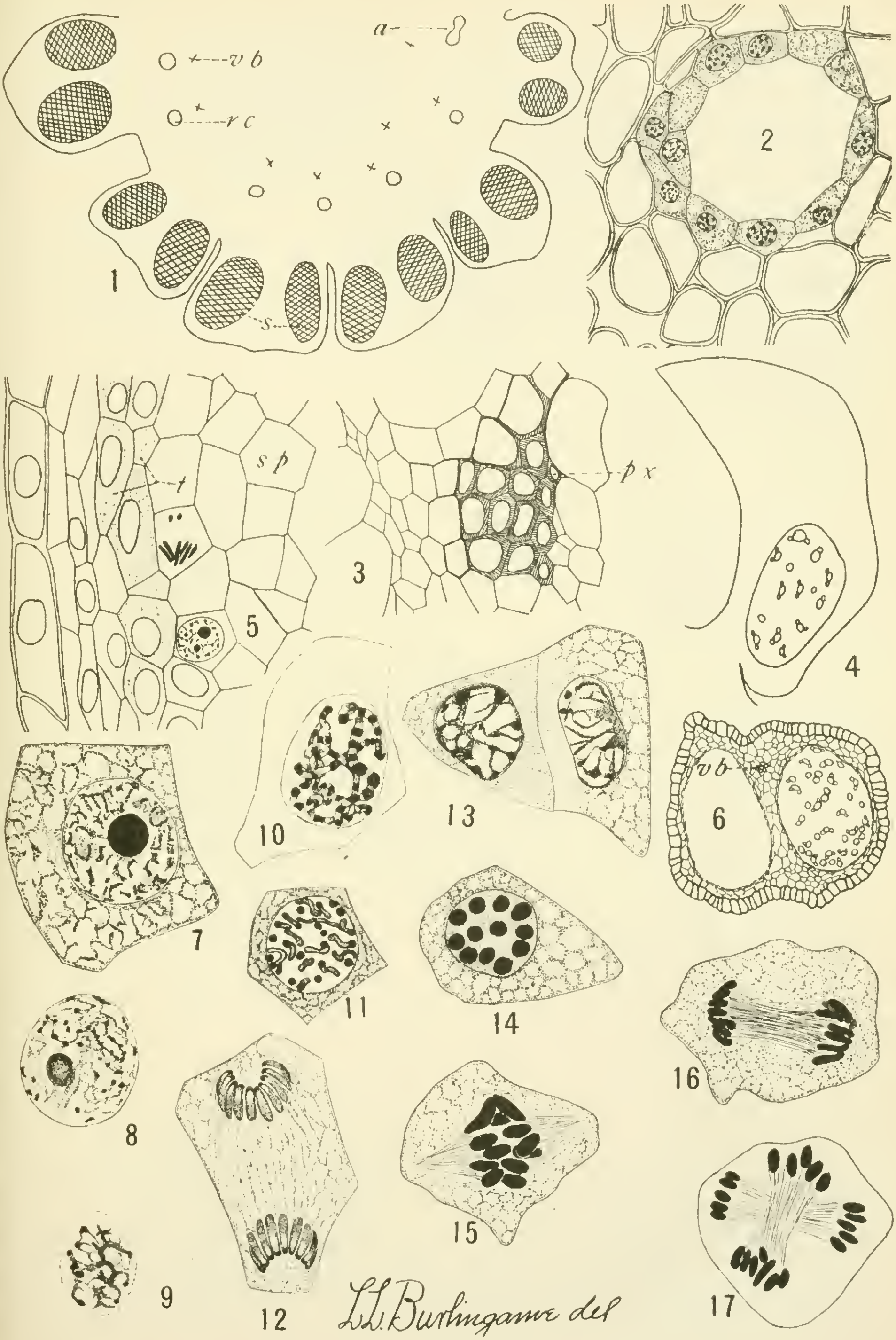
(*Figs. 1, 2, 5, 7–17* are *P.* sp.; *figs. 3, 4, 6* are *P. totarra Hallii*)

Fig. 1.—Cross-section through the middle region of a staminate cone, showing the relative position of sporophylls and sporangia (*s*), vascular bundles (*vb*), and resin canals (*rc*); the canals sometimes branch as shown at *a*.

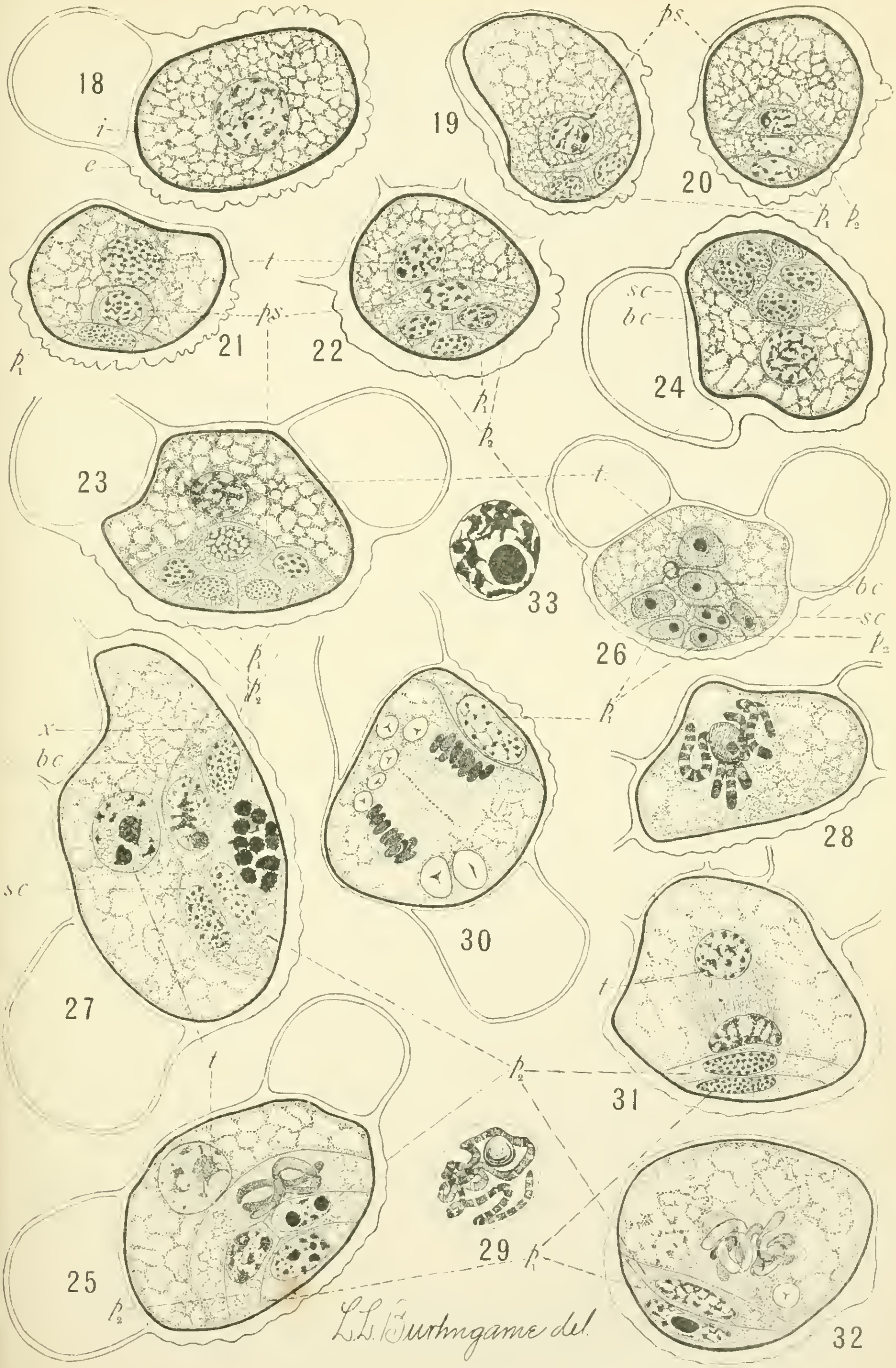
Fig. 2.—Cross-section of resin canal.

Fig. 3.—Cross-section of vascular bundle from strobilus; *px* shows position of protoxylem.

Fig. 4.—Longitudinal section of a sporophyll, and one sporangium of a young cone about 3^{mm} in length.



BURLINGAME on PODOCARPUS



BURLINGAME on PODOCARPUS

Fig. 5.—Detail of sporangium of same age as preceding, showing relations of wall, tapetum (*t*), and sporogenous tissue (*sp*).

Fig. 6.—Cross-section through sporangia; *vb*, vascular bundle.

Fig. 7.—Cell of the sporogenous tissue about one division short of the mother cell stage.

Fig. 6.—Detail of a nucleus a little later than preceding, showing the nuclear materials beginning to arrange themselves into threads with here and there deeply staining chromatin knots.

Fig. 9.—Surface view of a still older nucleus showing the strands more distinct and larger.

Fig. 10.—The definitely organized spirem with unusually distinct chromatic knots; the spirem is usually more homogeneous and stains more evenly.

Fig. 11.—A very thin section of a nucleus whose spirem is beginning to segment into chromosomes; compared with *fig. 7*, it shows the variation in size and shape of the sporogenous cells.

Fig. 12.—Longitudinal section of a cell in telophase showing about half of each group of chromosomes; the spindle is curved with the concavity toward the observer.

Fig. 13.—Showing the reconstruction of the daughter nuclei.

Fig. 14.—Mother cell showing the emergence of the chromosomes from the granular mass out of which the spindle arises; the granular materials are actually much denser than shown in this figure and nearly conceal the chromosomes.

Fig. 15.—Longitudinal section of the heterotypic spindle.

Fig. 16.—Late anaphase of the heterotypic mitosis.

Fig. 17.—The homotypic mitosis.

PLATE IX

(*Figs. 18–27, 33* are *P. totarra Hallii; figs. 28–32* are *P. nivalis*)

Fig. 18.—Section of microspore slightly oblique to the wings; *i*, intine; *e*, exine.

Fig. 19.—Male gametophyte showing first prothallial cell (p_1) divided, no second prothallial (p_2) cell, primary spermatogenous cell (*ps*), and in next section the tube nucleus (*t*).

Fig. 20.—Same stage as preceding except that there are here two primary prothallial cells (p_2), the second of which has divided (only one, p_2, of the segments shown).

Fig. 21.—Same as *fig. 19* except that the single primary prothallial cell (p_1) has not divided; *t*, tube nucleus.

Fig. 22.—Male gametophyte showing first prothallial (p_1) undivided, second $(p)_2$ divided once, primary spermatogenous cell (*ps*), and tube nucleus (*t*).

Fig. 23.—Same as preceding except that first prothallial cell (*p*) has also divided and the primary spermatogenous cell (*ps*) has sunk down between the derivatives of the second prothallial cell (p_2); adjacent sections show that there are here four derivatives of the second prothallial cell.

FIG. 24.—Showing the stalk (*sc*) and body (*bc*) cells as well as prothallial cells (p_1, p_2) and tube nucleus (*t*).

FIG. 25.—The mitosis giving rise to stalk and body cells from the primary spermatogenous cell.

FIG. 26.—A usual type of mature gametophyte, having four derivatives of the first primary prothallial cell (p_1), four of the second (p_2), stalk (*sc*), and body (*bc*) cells, and tube nucleus (*t*); nine cells also usual (two p_1, four p_2, stalk, body, tube).

FIG. 27.—Showing the prophase of the division of the first prothallial cell (p_1) and what appears to be a "cell" (*x*) on the side of the body cell opposite to the stalk cell (*sc*); no nucleus could be discovered in this "cell;" see text for further details.

FIG. 28.—Division of the microspore nucleus in *P. nivalis*; a peculiar segmented appearance of the spirem, which has about completed segmentation into chromosomes, is shown.

FIG. 29.—A similar but younger spirem which has not yet begun to segment as the preceding one has done.

FIG. 30.—Anaphase of the division resulting in second prothallial cell (p_2) and antheridium initial.

FIG. 31.—Late telophase of division of antheridium initial into primary spermatogenous cell and tube nucleus; this is the usual shedding stage of this species, with prothallials undivided.

FIG. 32.—Metaphase of division of antheridium initial showing the twelve gametophytic chromosomes; see also *fig. 27*.

FIG. 33.—Details of nucleus of primary spermatogenous cell of *P. totarra Hallii.*

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THE SEEDLING OF CERATOZAMIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 115

(WITH TWO FIGURES AND PLATES XII–XVI)

HELEN A. DORETY

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THE SEEDLING OF CERATOZAMIA

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(WITH TWO FIGURES AND PLATES XII–XVI)

The vascular anatomy of adult cycad stems is likely to be obscured by two sets of complications. One, called anomalous thickening, described by VON MOHL (14) in 1832 in species of Encephalartos, is limited to certain forms; the other, the phenomenon of girdling, was described by KARSTEN (5) in *Zamia muricata* Willd. in 1856, and by METTENIUS (8) in Cycas, Encephalartos, and Dioon in 1860. Since then it has been found in the three other genera investigated. In the seedling these complications are either absent altogether or are just being initiated, hence the selection of juvenile forms is probably even more important in the anatomical study of cycads than in that of any of the other vascular plants. Anatomists have recognized this and have given us numerous descriptions, only the more pertinent of which need be cited here.

In 1856 KARSTEN (5), in the work mentioned above, found that each of the cotyledons of Zamia receives only one bundle, which may branch; that the central cylinder is broken up by parenchymatous communication between pith and cortex; and that in older stems, with the increase in the number of cauline strands, there is a corresponding increase in the number of bundles entering the leaves, because every bundle of the central cylinder contributes a trace. He also observed the frequent anastomosing of the leaf traces reported by VON MOHL, as well as the occurrence of girdling.

In 1884 BOWER (1) wrote a few descriptive notes on the seedling of *Cycas Seemanni* Al. Braun. He found the two cotyledons unequal in size and dissimilar in vascular anatomy. He says that while in some there is a median bundle which extends the whole length of the cotyledon without branching, in others there are two equal bundles near the center.

In 1887 GREGG (4) reported anomalous thickenings in the root of this species.

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In 1896 WORSDELL (**16**) discovered them in the adult stem of *Macrozamia Fraseri* Miq., giving it an appearance which recalled to his mind the stem of the Medullosae.

In 1897 the same author (**17**) gave the name "transfusion tissue" to certain short, scattered tracheids with reticulate markings on their transverse walls, found in lateral communication with the bundles in the green parts of some gymnosperms. He considers this tissue a "direct derivative of the centripetal xylem" in the vascular bundles of fossils. Later in the same year (**18**) he recorded its occurrence in the cotyledons of *Cycas revoluta* Thunb. and in the leaf traces of *Stangeria paradoxa* T. Moore. He found that in each of the two cotyledons of *Cycas revoluta*, there are three collateral bundles, which may increase to five; that there may be some centrifugal wood near the base of the cotyledon, but none farther out; that the root may be tetrarch or triarch; that girdling had not yet begun in the young seedling he was investigating; and that anomalous thickenings were conspicuous. In *Stangeria paradoxa*, the two cotyledons have a common stalk, each is multifascicular, and the bundles are said to be concentric near the base. The root is triarch, changing to diarch near the tip. In the same paper, the two cotyledons of *Macrozamia spiralis* Miq. are said to be like those of *Cycas revoluta*.

The author calls attention to the absence of anomalous thickenings in this species of Macrozamia in contrast with the mature stem of *M. Fraseri* previously studied by him. In all three seedlings WORSDELL missed the transition from stem to root.

In 1898 the seedling of *Bowenia spectabilis* Hook. was described by PEARSON (**9**). He says that each of the two cotyledons has four to seven bundles derived from one, and that these bundles are all collateral with normal orientation. He is emphatic as to the collateral nature of the bundles, even after having examined a preparation of WORSDELL'S in which the latter considered them concentric. In the leaves the bundles are oriented normally, and the centripetal wood, scanty at the base, becomes more and more abundant farther up the petiole; but the centrifugal wood does not disappear even in the pinnae. The root is tetrach or pentarch, but may reduce to triarch. In the young material at his disposal, PEARSON found no anomalous thickenings in the root, but they were discovered later in presumably older plants by WORSDELL (**19**), who seized the occasion to emphasize the similarity to the Medullosae.

In 1904 MATTE (**6**) published his masterly thesis, which in the third part treats of the seedlings of *Dioon edule* Lindl., *Cycas siamensis* Miq., and *Encephalartos Barteri* Carruth. Of his two seedlings of *Dioon edule* one proved abnormal, one cotyledon being partly aborted. The cotyledonary bundles are mesarch near the base, but the protoxylem moves quickly outward, so that the bundles are exarch throughout most of their course. The axis is considered as an aggregation of leaf bases, and the girdling is explained as being due to intense intercalary growth produced under the influence of the developing leaf within. The four root poles are inserted upon the cotyledonary bundles, the median bundles furnishing insertion for two diagonally opposite poles, and each lateral trace uniting with its mate of the other cotyledon to furnish insertion for the other two poles. Mucilage canals are reported in the root, for the first time in a cycad. The root of *Cycas siamensis* is diarch; its two poles are inserted upon the median bundles of the cotyledons, the lateral bundles dying out in the cortex. Anomalous thickenings or cortical vascular strands are conspicuous; MATTE'S demonstration that these are not abnormal, but merely remnants of an ancestral character, justifies his objection to the term "anomalous" used to describe them.

In Encephalartos the root is pentarch, and the cotyledonary bundles—a single, ringlike trace from each of the three cotyledons—fuse with the leaf traces at different levels before entering the central cylinder. This cylinder is polystelic, as in *Medullosa anglica* Scott. In considering this feature, MATTE indorses WORSDELL'S view that it helps to relate the Cycadales to ancient forms like Medullosa rather than to monostelic forms like Heterangium as SCOTT suggests. He regards the root of cycads as a "new organ" inserted upon the lower extremity of the hypocotyl, and not merely an extension of that organ.

The main facts discovered by these investigations, and agreeing, so far as they relate to *Dioon edule*, Cycas, and Zamia, with unpublished studies made in this laboratory by LAND, THIESSEN, and others, may be classified as follows:

Characteristic features.—Under this heading may be placed the occurrence of two cotyledons, the hypogean character of germination, the thick tap root, centripetal wood in the cotyledons and leaves, girdling, and numerous mucilage ducts.

Character upon which the seedlings may be divided into two groups.—This is the presence or absence of anomalous thickenings or cortical vascular strands. They occur in Cycas, Bowenia, and Encephalartos; they are said to be absent in Zamia, Dioon, Macrozamia, and Ceratozamia, though they were found in the mature stem of *Macrozamia Fraseri* by WORSDELL. Microcycas remains to be reported upon.

Features peculiar to certain genera.—We may place here the single cotyledon reported for Ceratozamia, the three cotyledons of Encephalartos, concentric bundles in the base of the cotyledon of Stangeria, the polystele in the earliest formed part of the axis of Encephalartos, and mucilage ducts in the root of Dioon.

The present series of studies aims in the first part to add to the list a detailed account of Ceratozamia, Microcycas, *Dioon spinulosum*, and species of Zamia; and in the second part to extend the investigation to the conifers by examining some juveniles of each of the great groups. Podocarpus, Keteleeria, Cunninghamia, *Pinus edulis*, *P. Banksiana*, and a few others, as well as the cycads mentioned, are well under way.

Ceratozamia

In 1846 BRONGNIART (**2**) gave the generic and specific names to *Ceratozamia mexicana*, a "new cycad from Mexico." He described the adult forms and the ovulate and staminate strobili. In 1870 WARMING (**15**) reported the monocotyledonous character of the embryo. In 1872 VAN TIEGHEM (**13**) examined four seedlings and found a thick scale enclosed by the sheathing base of the single cotyledon, and, in turn, enclosing a hairy foliage leaf with circinate vernation. One of the four seedlings was suspected by him of having the rudiment of a second cotyledon. He describes the root after the xylem and phloem have reached their final position, and gives the number and derivation of the cotyledonary strands and early leaf traces; but in these respects, no two of the seedlings agree. A piece of mature stem, examined by SOLMS-LAUBACH (**12**) in 1890, was found to have a single cylinder, but peduncular strands occurred in the pith, an observation which was corroborated later by WORSDELL (**16**). In the work previously mentioned (**6**) MATTE made a careful study of the ovulate cone and the mature leaf, with a few observations on younger leaves. He found terminal teeth on some of the pinnae of the younger plants, and at the bases of the petioles stipules with a bundle in each. He says that the meriphyte of the leaves has a modified Ω-shape in cross-section, with an anterior system, and shows that in this as well as in other cycad petioles the so-called fusions of the traces are often only approximations (*accolements*).

MATERIAL AND METHODS

The seedlings of Ceratozamia and *Dioon spinulosum* were grown from seeds provided by Professor CHARLES J. CHAMBERLAIN; the Microcycas seedlings were given to me by Professor OTIS W. CALDWELL, and later some were grown from seeds secured by Professor CHAMBERLAIN. The Zamia seedlings were furnished by Dr. W. J. G. LAND.

Having in mind the danger of drawing conclusions from a few specimens, I have used freely the wealth of material at my disposal. The chief part of the investigation was made from material fixed in picro-acetic-alcohol, stained with safranin and anilin blue, and cut in serial sections. *Figs. 21, 22,* and *23* are diagrams of wax models constructed from serial sections.

GENERAL DESCRIPTION OF EMBRYO AND SEEDLING

The seeds of Ceratozamia are about 2.5^{cm} long and 1.5^{cm} thick, that is, about the same size as those of *Dioon edule,* and intermediate between *Dioon spinulosum* on the one hand and Zamia on the other. Like most cycad seeds, they are flattened on two or three sides by the pressure of growth within the cone, and it is not unimportant to remember that during the whole period of embryonal development —the longest known for a cycad—the seed lies upon whatever side it happens to fall. The single cotyledon begins to be differentiated in November, and early in December its appearance is like that represented in *fig. 1.* Later it begins to surround the axis (*fig. 2*), and finally the two edges meet (*fig. 3*). In these early stages, the coleorhiza is proportionally long; but later elongation of the cotyledons forces the base of the embryo backward, so that both coleorhiza and suspensor are crushed little by little to a flat, brown disk (*figs. 4, 5*). Further elongation forces the base of the embryo through the softer, micropylar portion of the seed coat; *figs. 6, 7, 8* show this and the two succeeding stages. In *fig. 7* the base of the cotyledon is curved downward, and the plumule is seen issuing from between its opened edges; and it may be observed that this method of development has thrown the first leaf out of alignment. The edges close in again, and remain adhering the full length of the cotyledon (*fig. 13*). The tip is sometimes lobed (*figs. 14, 15*).

The cotyledon always develops on the lower side of the embryo as the seed lies during germination. In seedlings which were turned after the cotyledon had begun to develop, the plumule has not succeeded after a year in emerging from underneath the cotyledonary sheath with which it is hampered.

Fig. 8 shows the appearance of the tardy root, which has made its way through the brown cap formed from the remains of the disorganized coleorhiza and suspensor. When the root pierces the soil, the starch is transferred to it from the endosperm, and the root thickens into a tap root. By its further penetration into the soil, it often draws the upper portion further down, imbedding the seed, and possibly giving to the first series of lateral roots their initial upward slant. The lateral roots almost always appear in threes, whether the root be tetrarch or triarch. *Fig. 9* represents a seedling toward the close of this period of its activity. The extreme shortness of the hypocotyl may be conjectured from the small distance between the base of the cotyledon and the insertion of the first whorl of lateral roots. The plumule is composed in this case of two brownish, hairy scales, enclosing a foliage leaf with circinate vernation (*fig. 10*). Each scale is terminated by a sharp, curved point. The number of scale leaves varies in different seedlings; some have only one, and in in some few observed the first organ was a perfect foliage leaf. The base of both scale leaf and foliage leaf is furnished with broad, winglike expansions which enclose the next leaf. The petiole of the first leaves reaches a length of 15–20^{cm}, and bears one or two pairs of pinnae inserted near the adaxial face. Between the two terminal pinnae there is a tiny, sharp spine, which has its counterpart in the apical point of the scale leaves. At irregular intervals along the petiole are other, even smaller points. The first leaves are opposite, but the later ones assume the spiral phyllotaxy represented in *fig. 12*.

ANATOMY

As was above noted, the edges of the cotyledon close in after the exit of the plumule, the two halves of the adaxial face meeting in a plane represented by the line *ad* in *fig. 13*. Three bundles enter the cotyledon from the vascular plate, and each dichotomizes again and again (*fig. 16*), the median one being no more a "double" bundle than any of the others. The number of traces may be increased to fifteen toward the upper portion, but is gradually reduced again toward the tip, some bundles approximating in pairs, others dying out. Those in the lobes disappear lower down than the others (*fig. 15*). The bundles are collateral throughout the cotyledon, and their orientation is normal, as seen in *figs. 13, 14, 15*, where the xylem faces the line *ad*. The wood is mesarch (*fig. 17*), with the protoxylem gradually moving out toward the phloem as it ascends. In the upper portion centrifugal elements are wanting, that is the wood is exarch (*fig. 18*). The vascular system of the cotyledon is differentiated relatively early in the development of the seedling. In that from which *fig. 19* was drawn, in which no root has yet appeared, the vessels are fully matured. WORSDELL'S transfusion tissue was frequently observed in direct continuation with the centripetal xylem (*fig. 18, tr*).

Mucilage canals are numerous in the lower part, and seem to bear no definite relation to the strands (*figs. 30, 31*); farther up they usually number one more than the strands and alternate with them; higher still they die out irregularly; in some cotyledons they are absent altogether.

The vascular plate of the hypocotyl axis is irregularly four-sided as seen in cross-section; all the xylem is in the center, sometimes in a solid mass, sometimes interspersed with pith cells. The protoxylem points are clearly distinguishable. In the plant from which *fig. 20* was drawn, the protostelic condition persisted through a vertical distance of 1.6^{mm}. Above this the pith cells occupy the central region, so that a siphonostele replaces the protostele (*fig. 28*). About

1^{mm} above the upper terminus of the protostele, the xylem is grouped sometimes in four conspicuous mesarch lobes, but oftener in three prominent ones and a fourth weaker one (*D*, *fig. 29*). From this weaker lobe a very small strand passes out (*D*, *figs. 21–24, 26, 30*), and in some cases it branches, but is lost in the cortex. From the lobe diametrically opposite, the median strand of the cotyledon (*C*, *figs. 21, 24, 25, 29, 30*) enters that organ; and from each of the other two angles or lobes a strand passes out (*A*, *B*) and branches, one member in each case bending tangentially (*b*, *b′*) to form the lateral trace of the cotyledon, the other, a very small strand (*a*, *a′*), either fusing with the leaf traces or dying out in the cortex. This cotyledonary node is represented in cross-section in the diagram (*fig. 24*).

Comparison with the same node in Zamia, Cycas, *Dioon eduel*, and Microcycas suggested that the smaller bundles (*D*, *a*, *a′*) were the mates of the larger ones on the side of the cotyledon (*C*, *b*, *b′*), and that the second cotyledon was suppressed. The cause of the suppression was indicated by the long-continued one-sided presentation to gravity during germination, and the fact that the cotyledon is always on the under side. It was with the intention of testing these surmises that the experimental work already recorded (3) was undertaken. *Fig. 25* represents the cotyledonary node in Ceratozamia embryos developed on the clinostat, and *fig. 13a* a transverse section of their cotyledons.

Although the stem contains several layers of extrafascicular cambium (*cb*, *fig. 32*), I have not been able to find in two-year-old plants any anomalous thickenings except the solitary bundle (*fig. 33*) whose position in the base of the cotyledon is indicated in *fig. 30* at *z*. This bundle has its origin in a small group of cells (*cb″*, *fig. 32*) in the outermost layer of cambium. It is about 0.4^{mm} long and approximately vertical. Its tracheids have only spiral thickenings.

The foliar bundles (*fb*) may occur in four groups alternating with the cotyledonary bundles (*figs. 21–23, 26, 30*); but oftener there are only three groups, because of the fusion of two of them or the entire elimination of one, on the side of the suppressed cotyledon, when the main bundle (*D*) is slight or entirely wanting (*fig. 26*). At first they are all vertical; higher up they branch, and a strand from each group is sent to the first leaf (*fig. 22*). As previously noted, this organ, whether scale leaf or foliage leaf, is displaced laterally by the peculiarities of its development. All the traces which enter it are likewise distorted, giving rise to a pseudo-girdling condition which is apparent in very young seedlings (*figs. 22, 27, 31*). The two traces which enter from the groups nearest the leaf (*e, e′*) take first a radial, and then a tangential course to reach a position in the middle of the leaf (*figs. 22, 31*), showing on the way a tendency to branch. The traces (*d, d′*) supplied from the groups farther away take a tangential course, each giving off vertical branches, which in turn branch again. The remaining traces of the original four foliar bundles ascend vertically (*fig. 25*), and branch and anastomose freely. Only a limited number, in most cases four or five, remain (*fb, figs. 24, 31*). Before reaching the leaf base, each of these divides, one member entering it (*t, fig. 23*), the other, which remains small, being directed toward the growing point of the stem (*u, figs. 23, 27*). *Fig. 23* represents the branching and anastomosing of these strands. It should be noted that the vertical scale of the three diagrams (*figs. 21–23*) is magnified considerably.

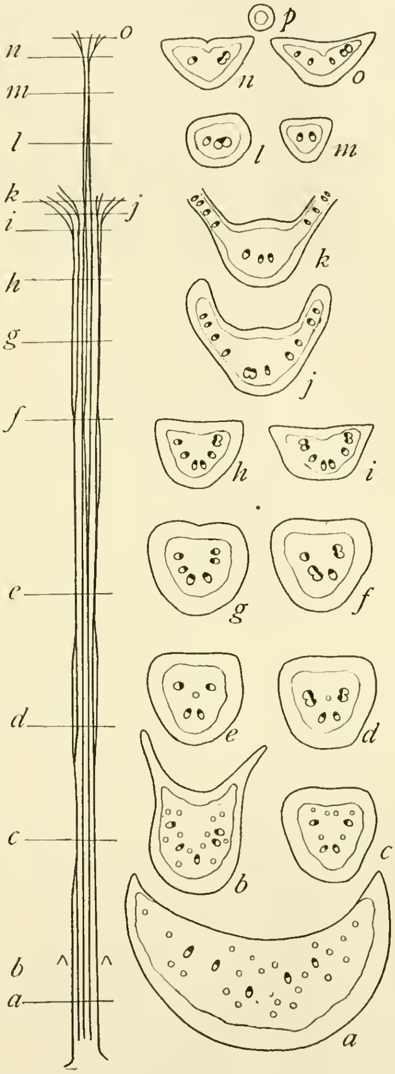

FIG. 1.—Study of foliage leaf from base (*a*) to apex (*p*); for explanation see text.

In the younger stages there is no attempt at girdling on the part of the leaf traces, except that which has been referred to in the first leaf as pseudo-girdling, whose cause is to be sought in the accident of its development. When the inner face of a leaf encloses a mass whose diameter is less than 3^{mm} (fourth leaf in *fig. 12*), the vascular bundles of that leaf are all vertical; but when the enclosed mass, by its enormous radial growth, has reached a diameter of $5–8^{mm}$ and comprises two or more developing leaves (second leaf in *fig. 12*) the base of the enclosing leaf enlarges its inner face accordingly. The first stage in the enlargement consists in an increase in the number of cells, but the second in a horizontal elongation of these (*text fig. 1*). While this is going on the older part of the central vascular cylinder is also increasing its diameter, separating farther and farther the original positions of the bundles of the leaf. As a result, the marginal traces gradually elongate as they are drawn more and more from the vertical position, and their upper parts stretch outward in the direction which the leaf takes. These facts have been observed repeatedly; whether they are an adequate solution of the problem of the cause of girdling, as MATTE thinks, I am unable to say.

In seedlings with three or four leaves, the stem bundles (*u, fig. 19*) branch repeatedly, and many of the branches reunite to form a small number of traces. Each of the remaining ones now forks once, the larger member in each instance going to the leaf as before, the smaller one continuing in the axis. Thus, even at this early stage, there is present the sympodial stem described by Miss SMITH (**11**) for older plants.

The number of strands entering successive leaves was seen to increase, sometimes with great regularity. In the plant whose dissection is represented in *figs. 11* and *12*, the number of traces was increased by one in each successive leaf, from the cotyledon with three, to the fourth leaf with seven; but the increment was not so constant in all the plants observed.

Within the leaf base and in the petiole, the bundles branch and anastomose freely. There is no real Ω in these first leaves; the bundles are arranged simply in an open arch. *Text fig. 2* is a study of a foliage leaf which was the second lateral organ of the plumule, that is, it was preceded by only one scale leaf. The scale leaf had four bundles; this leaf has five (*a, b*); but just above the stipules the number is reduced to four by the approximation of two of them (*c*).

Other changes occur as the meriphyte ascends the petiole (*c*, *d*). Just below the first pair of pinnae the branching is rapid, and at the level where the blade is seen exteriorly to separate from the petiole, there are four strands provided for it (*j*). In the rachis another approximation takes place (*l*), and the number of bundles is reduced to two (*m*). Each enters a pinna and branches continuously (*n*, *o*). The growing point terminating the rachis has no vascular trace in this leaf, in this respect agreeing with the young leaf represented in *fig. 10a*. As the plant increases in age, the meristem of this point retains its activity longer, producing a greater number of pinnae, as in the seed plants.

The wood in all the leaf traces is endarch in the central cylinder (*fig. 26*), but it soon becomes mesarch, with the protoxylem moving outward by almost imperceptible degrees. It never reaches the exarch condition, however; even in the tips of the pinnae there are always two or three elements of centrifugal wood. Here and there may be seen a few elements of transfusion tissue.

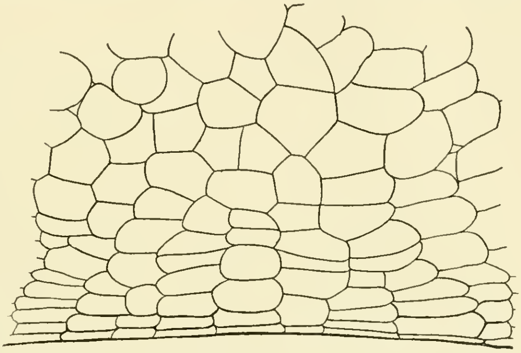
FIG. 2.—Cross-section of flattened cells from inner face of petiole near the base.

Mucilage canals are numerous in the leaf bases (*figs. 30, 31*), but they have no more definite arrangement there than in the base of the cotyledon. Throughout the region of the stipules there is a gradual decrease in number, which is not altogether due to fusing. Toward the upper levels of this region they are sometimes arranged in two series, an adaxial row and an abaxial one, with a definite relation to the bundles (*text fig. 2b*). About 1^{cm} above the base, there is left only one centrally located canal (*d*), which persists for half the length of the petiole (*e*).

The bases of the leaves as well as of the cotyledons are covered by a thick layer of cork. As was seen externally, the hypocotyl is extremely short, the transition from stem to root taking place in an almost horizontal plane (*fig. 30*). In the absence of cauline bundles, the root poles are inserted upon the cotyledonary strands (*A*, *B*, *C*, *D*, *figs. 34, 35*). There is no rotation of the protoxylem, but the phloem and metaxylem separate opposite the protoxylem groups (*Bn*, *Cn*, *fig. 36*), the cambium proliferating to fill the breach in the metaxylem, and the cortex invading the space left vacant by the phloem. The resulting halves swing, the one to the right, the other to the left, giving the appearance of a double fan of phloem and metaxylem connected by a single group of protoxylem. The right half of the phloem of one pole joins the left half of that of the next, and the cambium layer is thus curved inward (*cb*, *figs. 37, 38*). One result of this complicated process is that the entire xylem system of the root is bordered peripherally by cambium.

The medulla throughout the root is extremely meristematic, and its activity sometimes results in a displacement of one or more of the protoxylem groups, giving an unsymmetrical appearance in cross-section. This activity is indicated by the thin walls (*o*, *fig. 38*) showing recent division.

In all the plants I have investigated, the number of poles remains constant throughout any given root. This number may be three or four, depending seemingly upon the degree of development attained by the median bundle of the aborted cotyledon. *Figs. 26* and *34* will serve to illustrate this point. They represent the same level in two different plants. In the former the median bundle (*D*) was very weak, and there were but three root poles; in the latter this bundle was well developed and the root was tetrarch.

Fig. 39 represents the condition of the root tip in the region of the differentiation of protoxylem. The connective tissue is clearly defined, but there is no xylem plate connecting the poles. Examination of longitudinal sections of the root tip furnishes nothing that could be added to the description of REINKE (**10**). One initial group produces plerome, another periblem. There is no calyptrogen or dermatogen, but the outermost layers of the periblem become loosened at the tip and form the root cap (*k*, *fig. 42*).

There is no distinct endodermis, and the pericycle is several-layered, making it impossible to distinguish with absolute certainty any line of demarkation between phloeoterma and stelar tissue.

There are no anomalous thickenings in the roots of plants two years old.

In general, mucilage ducts extend but a slight distance into the root, usually about 8^{mm}, but in one specimen they were beginning to be formed in the plerome, immediately behind the region of its differentiation (*md*, *fig. 42*).

The lateral roots are diarch. *Fig. 40* represents a transverse section of a secondary root and the exit of a tertiary one. The uppermost lateral roots become ageotropic at a very early stage, and show symptoms of bacterial infection; and in some of the two-year-old plants, these roots present the characteristic "coralloid" appearance indicative of algal infection.

The root, as was seen above, is late in developing. *Fig. 27* represents the position and form of the meristematic plate, which has given rise to stem above, but is yet inactive below. *Fig. 19* shows the beginning of the activity which produces the root; the central portion of the active region in this figure is sketched in greater detail in *fig. 41*. In the stage represented in *fig. 42*, the root cap is developed and the plerome and periblem initial groups are easily distinguishable, but the plerome has not yet differentiated any xylem elements.

While in older seedlings the vascular systems of stem and root seem to be continuous, one sometimes finds sections which indicate a certain amount of interruption. Such a case is shown in *fig. 43*.[1]

Discussion

The result of the experimental work on Ceratozamia places this genus in line with the other cycads with reference to the dicotyledonous character. The proof of the abortion of the second cotyledon and the discovery of the manner in which that abortion was brought about naturally revive the question of the monocotyledonous nature of certain dicotyledons. It is possible that in some cases this condition may be caused by the same factor which produces it in the embryo of Ceratozamia, but this is not so in all cases; the experimental work now in progress upon these forms shows that other factors are involved.

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[1] When this investigation was completed, MATTE (7) published a preliminary note on the same subject. He says that the petiolary bundles have the Ω arrangement, that there is an anterior fascicular system in the region of insertion of the rachis, and that the pinna has terminal teeth. These features do not appear in any of my seedlings. Speaking of the root, he says it is tetrarch, and that he has observed a progressive reduction of poles, to three and even to two. As I have observed, no reduction occurred in the roots of any of the seedlings I sectioned, and the root is triarch as often as it is tetrarch. He has also found the abnormal thickenings strongly developed; but he does not give the age of the seedlings which manifest them.

The lobing at the tip of the cotyledon is suggestive of a primitive condition, which will be discussed in connection with *Dioon spinulosum*.

It would be interesting to compare the cotyledonary node in all cycads, in order to determine whether they are modifications of the same type or whether there are different types; but it is a matter of regret that all investigators have not considered it of sufficient importance. MATTE has described it fully for *Dioon edule* and *Cycas siamensis*. The latter is clearly a modification of the type which may be represented by the former. The present paper shows that Ceratozamia conforms to this type, and it may be in place here to say that Microcycas and the species of Zamia I am investigating are the same. WORSDELL'S *Cycas revoluta* and *Macrozamia spiralis* are doubtless similar. *Encephalartos Barteri* according to MATTE'S description, *Zamia muricata* according to KARSTEN'S, and *Bowenia spectabilis* according to PEARSON'S seem to differ from this type, in receiving only one bundle from the central cylinder; but there are two facts which conspire to make us consider that the seedling of Encephalartos described by MATTE was an unusual one: the presence of three cotyledons, and the union of the cotyledonary strands with the central cylinder at different levels.

That cycads, especially such fernlike ones as Stangeria and Bowenia, should be found to have an occasional concentric bundle is only to be expected from the nature of their fern origin. WORSDELL'S announcement, then, of such bundles in the base of the cotyledon of Stangeria was not a surprise, even though his drawings were not convincing. But the emphatic statement of PEARSON that he could not be convinced of it in WORSDELL'S preparation of Bowenia raises the doubt whether he would have recognized it in Stangeria. Of course the whole question resolves itself into the possibility of determining phloem in the absence of sieve plates.

In the feature of anomalous thickenings, as in so many other features, Ceratozamia appears to hold an intermediate position. The extrafascicular cambium is clear and distinct, yet in plants with two scale leaves, two expanded foliage leaves, and two or three leaves developing, the secondary fascicular systems are entirely wanting. The failure or delay of this cambium to function indicates that it is a vestigial character. I have seen it in Zamia as clear and distinct, though not so abundant, as in Ceratozamia.

There seems to be great variation in the number of root poles throughout the whole group of cycads and even in individual roots. This variation, taken in conjunction with the fact that in Ceratozamia the number depends upon the degree of development of the median bundle of the aborted cotyledon, indicates that this character is not to be depended upon as a phylogenetic one.

Summary

1. Ceratozamia is dicotyledonous, the second cotyledon being aborted by gravity.

2. The cotyledon is often lobed at the tip. It is multifascicular, and all the bundles are derived from three. The wood is mesarch at the base and exarch in the upper portion. Mucilage ducts usually alternate with the bundles.

3. The leaf traces are at first vertical; girdling follows upon increase in radial growth of the enclosed leaves and stem apex. The wood of the leaf traces is endarch in the central cylinder, but becomes mesarch in the leaf base and remains so to the tips of the pinnae.

4. The scale leaves are aborted foliage leaves.

5. The first-formed portion of the central vascular cylinder may be a protostele.

6. The stem is a sympodium.

7. There are several layers of extrafascicular cambium, but in seedlings two years old only the slightest trace of anomalous thickening.

8. The root is a delayed organ, and its four poles are inserted upon the cotyledonary bundles.

9. The entire xylem system of the root is bordered peripherally by cambium.

Grateful acknowledgments are due to Professor JOHN M. COULTER and Dr. W. J. G. LAND, under whose direction the investigation was conducted, and to Professor CHARLES J. CHAMBERLAIN for abundance of material.

THE UNIVERSITY OF CHICAGO

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EXPLANATION OF PLATES XII–XVI

All the drawings except *figs. 6–11, 16, 21–25* were made with the aid of a camera lucida. The abbreviations used are as follows: *A, B, C, D*, the four main cotyledonary bundles; *An, Bn, Cn, Dn*, the corresponding root poles; *C*, median bundle of developed cotyledon; *D*, median bundle of aborted one; *a, a'*, lateral bundles of aborted cotyledon; *b, b'*, lateral bundles of developed one; *ad*, adaxial face of cotyledon; *c*, cotyledon; *cb*, cambium; *cb', cb''*, extrafascicular cambium; *cl*, coleorhiza; *ct*, connective tissue of root; *cx*, cortex; *cfx*, centrifugal xylem; *cpx*, centripetal xylem; *d, d'*, lateral traces of first leaf; *e, e'*, middle traces of same; *f*, foliar bundles; f^1, f^2, f^3, f^4, four principal groups of foliar bundles; *g*, cotyledonary trace; *h*, vascular system of hypocotyl; *i*, vascular system of root; *imd*, initiation of mucilage duct; *j*, sclerenchyma; *k*, root cap; *l*, leaf; *m*, meristematic plate; *md*, mucilage duct; *n*, medulla; *o*, indices of radial cell division in medulla; *p*, plumule; *ph*, phloem; *pph*, protophloem; *px*, protoxylem; *px'*, rupture of tissues caused by protoxylem; *q*, plerome; *r*, root; *s*, suspensor; *t*, leaf trace; *tr*, transfusion tissue; *u*, stem bundles; *v*, periblem; *w*, stipules; *x*, xylem; *y*, cork; *z*, anomalous thickening.

FIGS. 1–5.—Stages in intraseminal development showing single lateral cotyledon and gradual enlargement of its base to encompass axis. ×5.

FIG. 6.—Rupture of micropylar end of seed coat and protrusion of base of embryo. ×½.

FIG. 7.—Bending of base of embryo and exit of plumule. ×½.

FIG. 8.—Appearance of root. ×½.

FIGS. 9, 10.—Young seedlings. ×½.

FIG. 10*a*.—Longitudinal section through tip of unfolding leaf. ×5.

FIG. 11.—Dissection of aerial portion of two-year-old seedling. Nat. size.

FIG. 12.—Diagram showing spiral phyllotaxy of later leaves. Nat. size.

FIG. 13.—Transverse section of cotyledon. ×8.

FIG. 13*a*.—Transverse section of cotyledons of embryo developed on clinostat. ×8.

FIG. 14.—Transverse section of upper portion of cotyledon showing tendency to lobe. ×8.

FIG. 15.—Transverse section of same cotyledon nearer tip. ×8.

FIG. 16.—Diagram to show venation of cotyledon.

FIG 17.—Mesarch bundle from lower portion of cotyledon. ×380.

FIG. 18.—Exarch bundle from the middle region of cotyledon. ×380.

FIG. 19.—Longitudinal section of lower part of embryo emerging from seed. ×8.

FIG. 20.—Transverse section of the vascular plate of hypocotyl. ×225.

FIG. 21.—Diagram representing rise of cotyledonary bundles from vascular plate.

FIG. 22.—Diagram showing origin and course of first leaf traces.

FIG. 23.—Diagram showing origin and behavior of traces of second leaf.

FIG. 24.—Horizontal diagram of cotyledonary node.

FIG. 25.—Same node in plants developed on clinostat.

FIG. 26.—Transverse section slightly above cotyledonary node showing the four main cotyledonary bundles and the three groups of foliar bundles. ×60.

FIG. 27.—Longitudinal section of embryo somewhat less developed than the one represented in *fig. 19.* ×10.

FIG. 28.—Transverse section of stele of hypocotyl 2^{mm} above the section represented in *fig. 20.* ×225.

FIG. 29.—Transverse section of stele of hypocotyl immediately below cotyledonary node. ×225.

FIG. 30.—Transverse section of plant with median bundle of aborted cotyledon fairly well developed. Same level as *fig. 26.* ×8.

FIG. 31.—Section of same plant 140 μ above that represented in *fig. 30.* ×8.

FIG. 32.—Detail of portion of axis 0.2^{mm} below *fig. 30*, between the cotyledonary bundle *B* and the phellogen; shows group of cambium cells which gave rise to anomalous strand *z*. ×75.

FIG. 33.—Transverse section of the anomalous strand represented in base of cotyledon in *fig. 30.* ×135.

FIG. 34.—Cotyledonary node of plant with median bundle of aborted cotyledon well developed. ×150.

FIG. 35.—Stele of hypocotyl 0.36^{mm} below *fig. 34*; xylem and phloem collected in ring. ×150.

FIG. 36.—Section of stele of same plant 0.16^{mm} below *fig. 35*; shows breaking apart of phloem opposite the protoxylem. ×150.

FIG. 37.—Same, 0.2^{mm} below the previous section; separation of metaxylem. ×150.

FIG. 38.—Section of root stele between the third and fourth whorls of lateral roots. ×179.

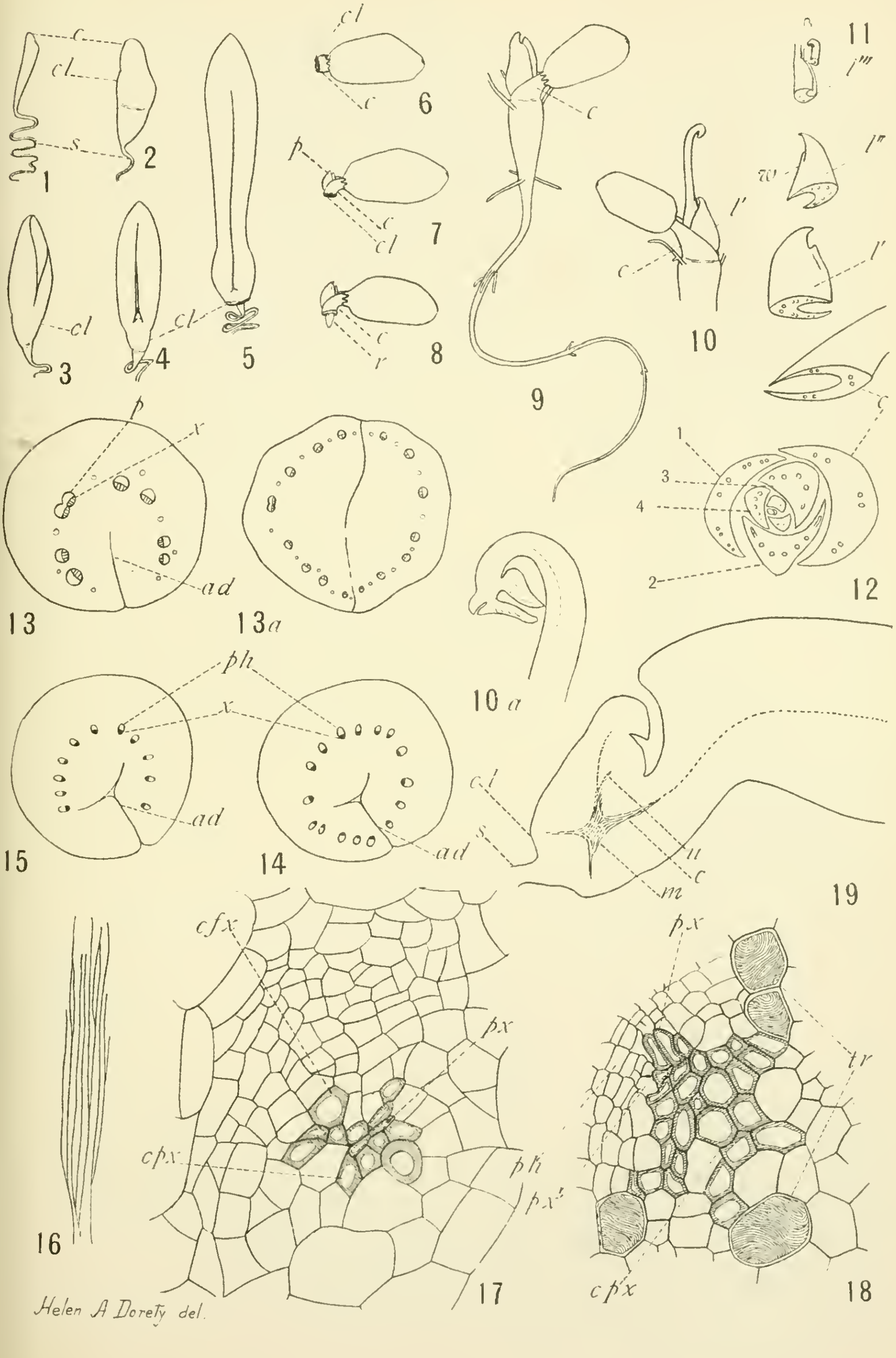
FIG. 39.—Section of stele near root tip at beginning of differentiation of xylem. ×225.

FIG. 40.—Transverse section of stele of lateral root showing exit of a tertiary root. ×225.

FIG. 41.—Longitudinal section of seedling showing beginning of root formation. ×10.

FIG. 42.—Detail of beginning of root formation. ×60.

FIG. 43.—Detail showing interruption between vascular tissues of hypocotyl and root. ×120.



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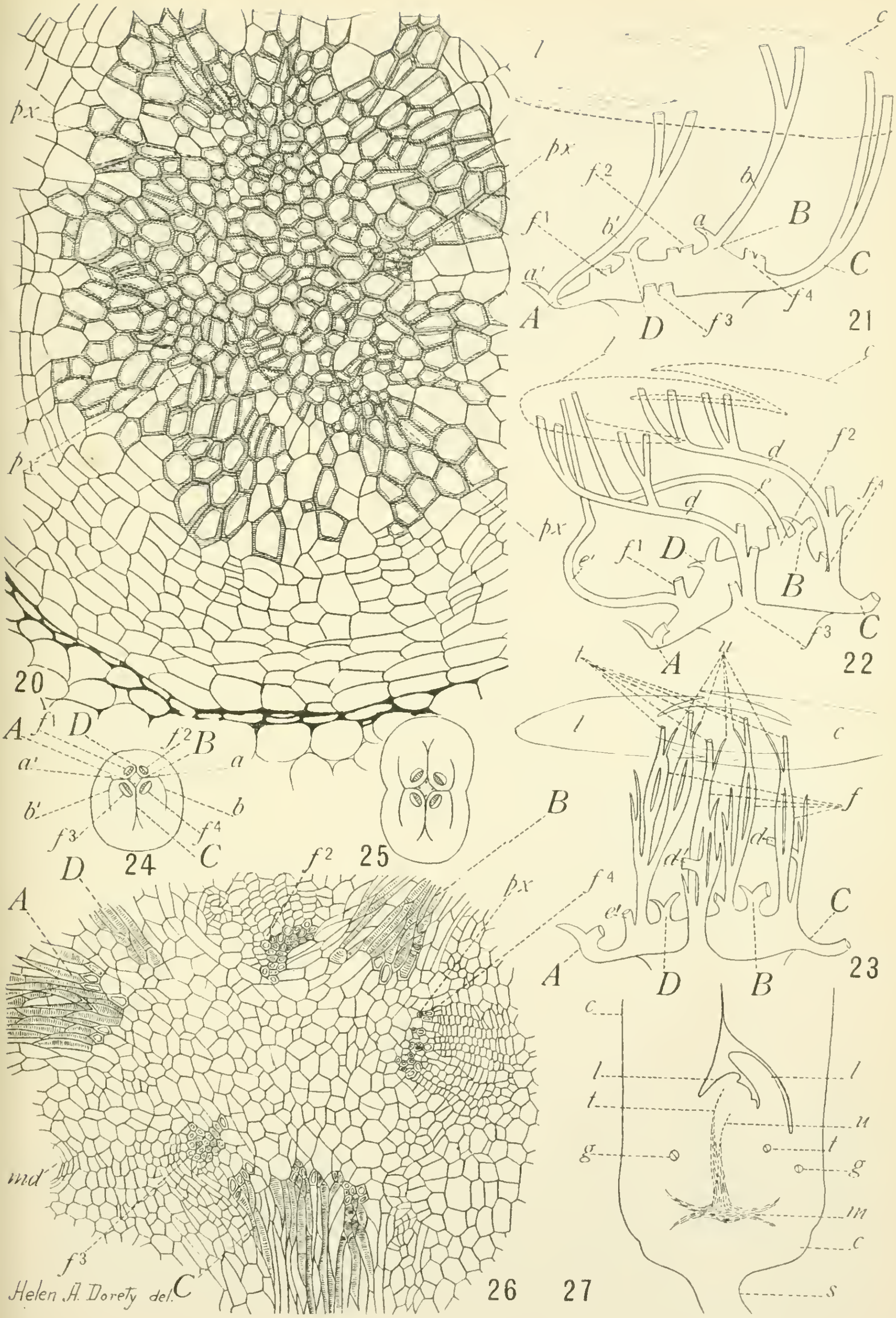
DORETY on CERATOZAMIA



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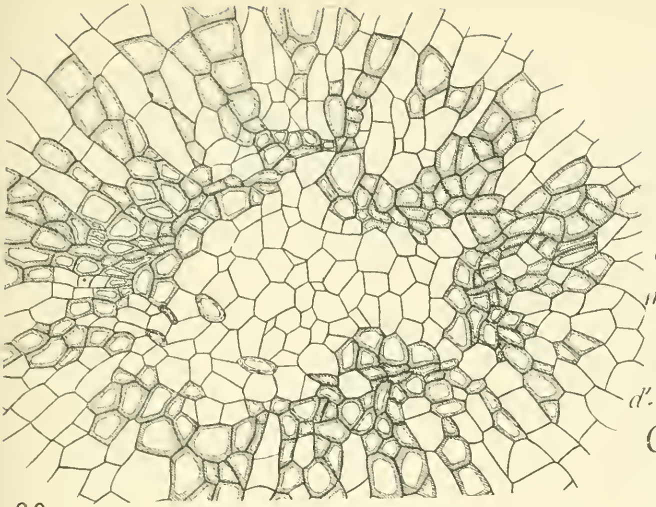

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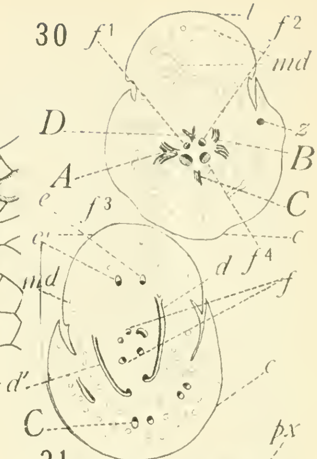

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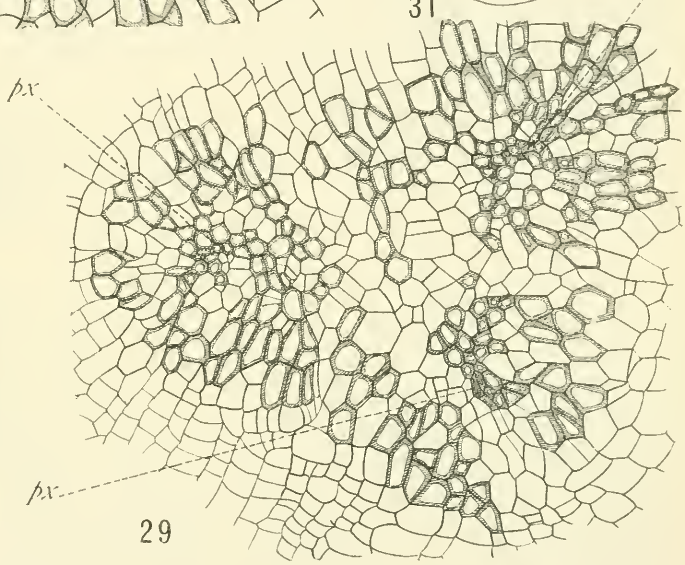

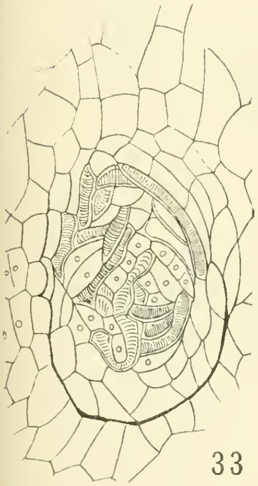

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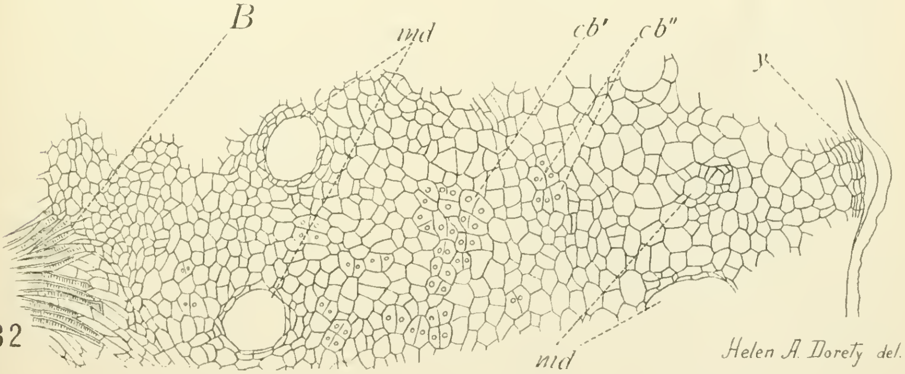

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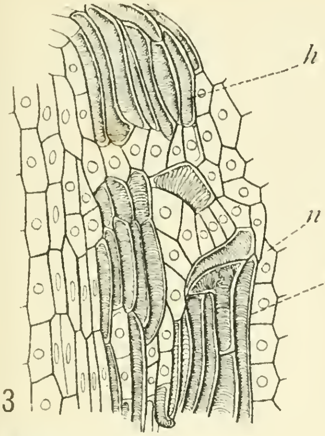
DORETY on CERATOZAMIA



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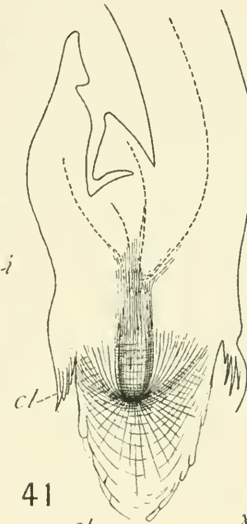

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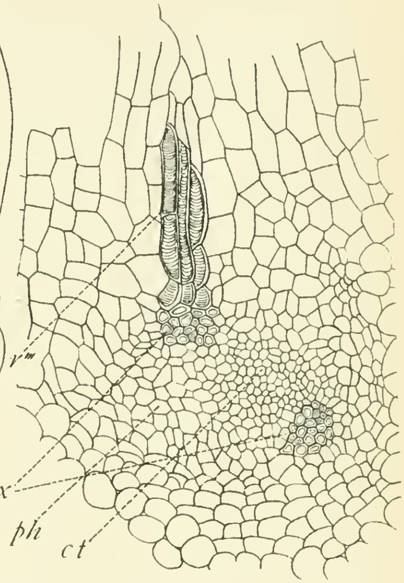

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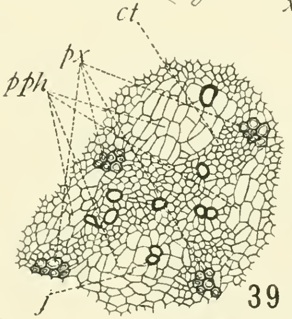

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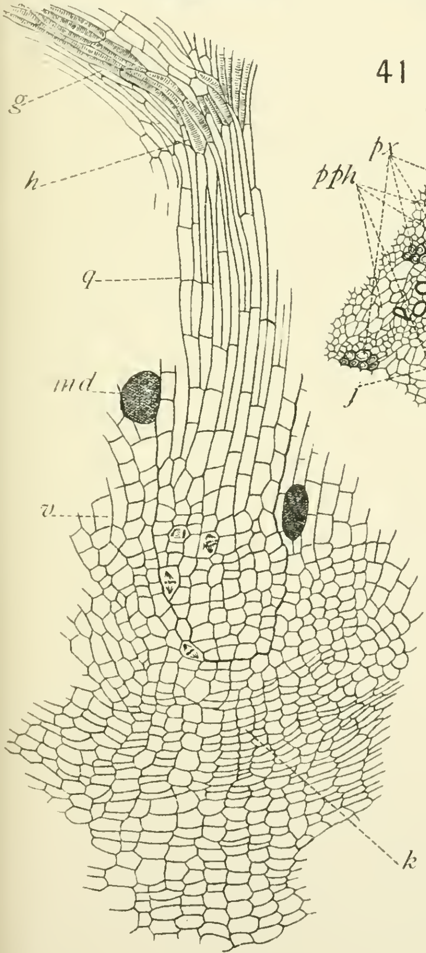

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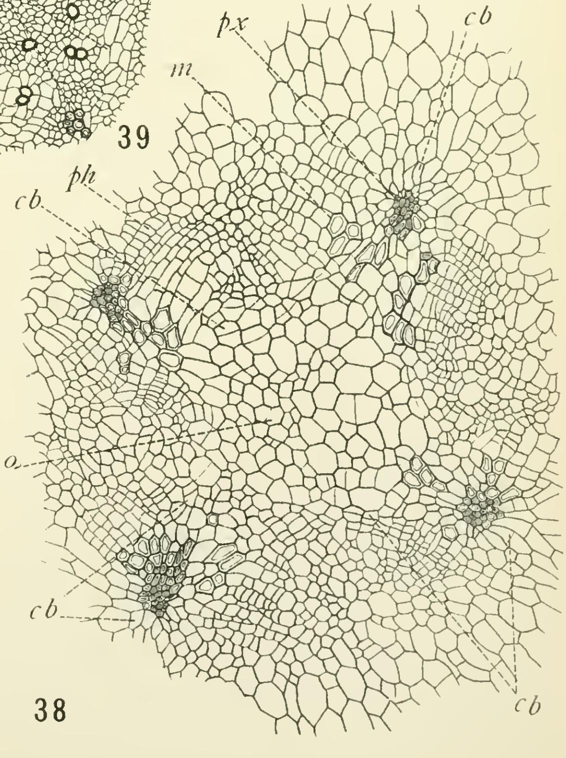

38

DORETY on CERATOZAMIA

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EFFECT OF ILLUMINATING GAS AND ETHYLENE UPON FLOWERING CARNATIONS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 116

(WITH FOUR FIGURES)

WILLIAM CROCKER AND LEE I. KNIGHT

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(WITH FOUR FIGURES)

1. Historical

As early as 1864 observations were recorded on the effect of illuminating gas on vegetation. Girardin (1) called attention to the phenomena of gas injury to trees as reported from various places in Rouen, Berlin, Hamburg, Hanover, etc. He especially investigated injury done to Italian poplars which had come into use as shade trees along the highways. He made a chemical analysis of samples of soil taken three feet from leaks in the gas pipes, and found inflammable oil as well as sulfur and ammonia compounds present R. Virchow (2) expressed an opinion that coal gas is especially injurious to vegetation. Kny (3) was one of the first to test the injury experimentally. He used three sound trees in the Berlin Botanical Garden, each about twenty years old—one maple and two lindens. Gas pipes were carefully laid 84^{cm} deep and the gas used was freed of sulfuretted hydrogen. The two pipes were laid in a circle about the maple, and four burners were attached at a distance of 118^{cm} from the trunk. Near each linden tree were two burners, 110^{cm} from the trunks. The gas escape was measured daily.

(1) Maple received daily	12.9 cubic meters
(2) First linden	11.7 cubic meters
(3) Second linden	1.6 cubic meters

The experiment was begun July 7 and lasted for (1) and (2) a half-year, for (3) a full year. First a Euonymus (*E. europea*) near the maple died, then the maple lost its leaves (September 1). At the same time an elm near by showed injury. September 30 the first linden showed signs of injury. On October 12 the first linden lost its leaves, and on October 19 the second, while other lindens in the garden were yet green. An examination of roots one-half inch in diameter showed a blue coloration extending out from the middle

JUN 13 1921

toward the periphery. The following spring the maple, elm, and Euonymus bush showed no signs of life. The lindens produced foliage, but the leaves were bleached and smaller than usual. Dried cambium and a rich growth of fungi were further indications of injury.

Similar investigations were carried on by SPÄTH and MEYER (4). In one case during the summer a little less than $1^{\text{cu. m}}$ of gas diffused daily through $17.8^{\text{cu. m}}$ of soil in a wooded plot. The roots of all the trees were killed within a few days. During four winter months the same amount of gas was allowed to escape into a wooded plat of twice the above area. In this case Platanus, silver poplar, American walnut, and Ailanthus were killed; the maple and horse chestnut were greatly injured; while the linden showed no injury. In another experiment $0.0185^{\text{cu. m}}$ of gas was daily distributed equally among seventeen trees. The experiment lasted from April 11 to June 27. Before May 30 six of the more sensitive trees had died. By June 21 all the others, with the exception of the rough-fruited maple, had slackened their growth. The leaves of the injured trees were a pale green or yellow, and most of the younger roots were dead. According to the statement of the gas inspectors, their methods were not capable of detecting such light leaks as are shown in this experiment. These investigators found that when the surface of the soil is compact, the gas may travel long distances before reaching the surface. An instance is cited of gas traveling from a leak on one side of a street to a cellar on the opposite side, where it became evident by an unbearable smell. These investigators concluded that trees are far less sensitive to gas leaks during the winter than during the growth period. They also found much more rapid injury where the surface of the soil was packed. The small quantity of gas necessary to kill and the great distance that gas travels through the soil serve to emphasize the danger to which trees are exposed.

H. EULENBERG (5), besides summarizing the results given in previous literature, adds the birch to the list of less sensitive trees.

J. BÖHM (6) grew slips of water willow in water through which gas was passed. He found that they produced only short roots and that these soon died, as did also the buds. The twigs themselves remained alive for about three months, until, as he believes, the reserve food had been exhausted. In another experiment he found that soil impregnated with gas was very poisonous to plants, for seeds put to germinate in it started, but their roots soon died. A Dracaena planted in such soil died in ten days. Far less injury was shown when a given quantity of gas was in contact with the portions of the plant above the ground than when the same quantity came in contact with the roots by being passed into the soil. The roots, he concludes, are most sensitive to gas injury. He found potted plants of Fuchsia and Salvia only moderately sensitive to illuminating gas that was allowed to bubble through the soil.

LACKNER (7) states that camelias, azalias, cacti, and ivy are much injured if kept in rooms where illuminating gas is burned; while palms, dracaenas (*Acuba japonica*), and many other plants escape uninjured. He asserts that it remains to be determined whether it is escaping portions of unburned gas or products of incomplete combustion that produce the injury.

C. WEHMER (8) calls attention to a severe case of gas poisoning in Hanover. Thirteen elm trees along a street showed injuries varying with the distance they stood from a leak in a gas pipe. In late winter a number of them showed brown discoloration of the inner bark, and a falling-off of bark in large patches extending up the trunk six feet from the ground. No blue discoloration of the roots appeared as was reported by KNY and other observers. The author asserts that the area of the injury was especially great because of the hard-packed street above the leak.

MOLISCH (9) found that illuminating gas is more injurious to the roots of plants than chlorin or carbonic acid. Growth in length is retarded by 0.005 per cent. of illuminating gas. If uninjured and decapitated roots of corn are grown in illuminating gas, the former are remarkably bent and retarded in their growth in length, while the latter grow almost straight and are comparatively vigorous. Under the influence of the gas the growth in thickness of the roots is increased, the greatest thickening occurring where the bending is sharpest. When a 10–20 per cent. mixture of illuminating gas exerts a stimulus from one side, the roots respond negatively.

NELJUBOW (**10**) notes some very interesting effects of illuminating gas upon the etiolated seedlings of peas and other legumes. WIESNER had already reported a horizontal nutation of these seedlings, which he explained as autonomic. RIMMER later explained this horizontal growth as a response to unfavorable conditions, especially lack of moisture in the air. NELJUBOW found that while this response always occurred in the dark in the laboratory air, it did not occur in the dark in a greenhouse or in the outside air. After determining that temperature and moisture were not factors, he sought the explanation in impurities of the laboratory air. He found that laboratory air passed through KOH, $Ba(OH)_2$, $CaCl_2$, red hot CuO, and finally through $Ba(OH)_2$ gave vertical seedlings; while similar treatment with the CuO unheated gave seedlings with the horizontal placement. This proved that some impurities (probably some of the constituents of illuminating gas) of the laboratory air, which were oxidized by glowing CuO, caused this peculiar horizontal placement. He later produced the effect with mixtures of illuminating gas. He likewise tested a number of the constituents of illuminating gas. Acetylene produced this nutation, but was difficult to work with, because, on the one hand, a slight increase in concentration killed, and on the other, it rapidly disappeared because of its high solubility in water. One part of ethylene in 1,000,000 of air gave the response, while one part in 4000 killed the majority of the seedlings. He likewise mentions the fact that various other constituents (benzene, sulfur dioxid, hydrogen sulfid, and carbon bisulfid) of illuminating gas are highly toxic. He makes no attempt, however, to determine the toxic limits of the several constituents, or to learn whether one or several determine the toxic limit of illuminating gas.

SHONNARD (**11**) mentions several manifestations of the injury of illuminating gas to trees, and describes an experiment with a potted lemon tree exposed to a flow of $1.07^{\mathrm{cu. ft}}$ of gas per hour. After eight days he notes the exudation of sap in considerable quantity from trunk and branches, as well as the discoloration and falling-off of the leaves.

RICHARDS and MACDOUGAL (**12**) tested the effect of carbon monoxid and illuminating gas upon various seedlings. Carbon monoxid, heretofore considered neutral, was shown to be toxic. It was not so effective as illuminating gas, however, in modifying the rate and amount of growth of root and shoot, in retarding the differentiation of the primary tissue, and in hindering the formation of chlorophyll. Gametophytes of certain mosses were found to be very resistant, suffering very little injury in high concentrations of these gases for three months. A more delicate moss, presumably *Mnium undulatum*, however, showed deleterious effects earlier. In Elodea and Nitella older cells were most injured, and the injury was shown by plasmolysis of the cells. A considerable part of their experimentation with illuminating gas serves to confirm the results obtained by MOLISCH, NELJUBOW, BÖHM, and others. The conclusion that "illuminating gas affords, in addition to the action of carbon monoxid, the results of the action of other substances deleterious to plants" seems to indicate that the work of NELJUBOW and others was entirely overlooked.

STONE (**13**) calls attention to the fact that very small leaks (2–3$^{\text{cu. ft}}$ per day) of gas may cause local injury to trees. Among manifestations of gas-killing in trees, he notes the early appearance of an abundant growth of fungi in contrast to the relatively late appearance on other dead trees. In speaking of the distance gas may travel he says: "In gravelly soils we have known gas to travel 2000 feet without difficulty, when the ground is frozen, and escape into the cellar of a house; whereas in heavier soils gas is more likely to be restricted to smaller areas."

RICHTER (**14**) and other investigators have pointed out a number of effects of impurities of laboratory air upon the responses of seedlings. RICHTER believes that in a number of cases the negative geotropism of hypocotyls is greatly weakened by these impurities. He points out that a one-sided illumination will produce far more nearly a horizontal position with than without these impurities. He likewise asserts that in many species the degree of horizontality from one-sided illumination indicates the degree of impurity of the air. He found great variation, however, in sensitiveness in different species even of the same genus.

2. Scope, method, and preparation of material

It is quite commonly asserted that plants do poorly in houses lighted with gas and especially is the flowering interfered with. Various inquiries have come to us from carnation growers as to the effect of illuminating gas upon the flowering carnation. These growers claimed to have had heavy losses from gas that seeped from defective pipes through the ground into the greenhouses. In some cases it is claimed that the losses occurred during cold weather, when little ventilation was possible and when the ground was frozen, so that upward diffusion from the defective pipes was hindered and thereby lateral diffusion fostered. In all these cases it is claimed that the injuries ceased with the repair or removal of the pipes.

Upon looking up the literature it was found that no accurate determinations were made upon the effects of illuminating gas and its constituents upon flowers, and that in no case have the toxic limits and relative toxicity of the several main constituents been determined. In short, it is not known in any case whether the toxic limit of the gas is determined by the action of one constituent or by the combined action of several. To answer these questions is the purpose of the investigation here reported.

This paper will deal entirely with the buds and flowers of the carnation, describing in detail the effects and toxic limits of illuminating gas and ethylene. A later paper will give in detail similar data for the other main constituents of illuminating gas, as well as describe the effects of illuminating gas and all its main constituents upon the vegetation of the carnation. The work naturally falls into these two divisions, for, as will be shown by experiments described later, the flowers are far more sensitive to illuminating gas than is the vegetation, and the toxic limit of the gas on the flowers seems (from all the evidence of our experiments) to be entirely determined by the ethylene it contains.

To determine the relative sensitiveness of buds and flowers on the one hand, and the vegetation on the other, as well as the relative sensitiveness of buds and flowers of different ages, one series of experiments was carried on by exposing entire potted plants to an atmosphere containing small proportions of gas. This was done by setting the plants into an air-tight greenhouse within the laboratory greenhouse, and then running desired quantities of gas into the air-tight greenhouse. This sort of experiment has some serious faults. It does not determine whether the flower is affected directly by the gas contained in the air about it, or whether the effect is indirect by injury to the plant through the absorption of gas by the soil and later by the roots. Also no definite determination of the toxic limit of the gas can be made, for the amount absorbed by the soil is not determinable.

To avoid such sources of error the buds and flowers still intact were exposed individually to the desired concentrations of the gases. This was accomplished by the use of the apparatus shown in *fig. 1*.

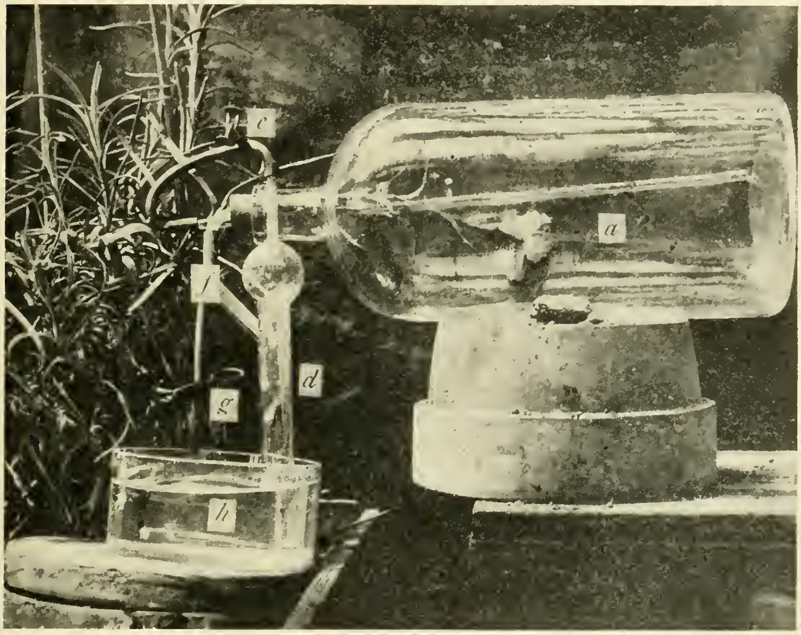

FIG. 1.—For description see text.

The bottle *a* is furnished with a three-holed rubber stopper. In one hole of the stopper is a straight glass tube reaching nearly to the bottom of the bottle. A calcium chlorid tube (*d*) is attached to the projecting end of this tube by means of a rubber tube furnished with a pinchcock (*e*). In the second hole of the stopper is a short bent glass tube (*f*), the outer end of which is furnished with a rubber tube and pinchcock (*g*). The third hole in the stopper is small and is capable of having the stem of the carnation inserted from the side by a split, which reaches from the hole to the margin of the cork. In setting up the experiment the cork is placed on the stem of the carnation by opening the cork at the split and inserting the stem. The flower or bud and the long tube are put into the nozzle of the bottle and the cork forced in until the whole apparatus is air-tight. The free end of the calcium chlorid tube is placed into a dish (*h*) of water or (with gases highly soluble in water) mercury; both pinchcocks (*e* and *g*) are opened; and suction applied to the tube *f* until the liquid rises to the small portion of the calcium chlorid tube, at which time pinchcock *g* is closed. The desired quantity of gas which is now poured into the wide end of the calcium chlorid tube rises to the top of the liquid. A one-holed rubber stopper, furnished with a tube and attached to a column of the same liquid as is contained in the dish, is now inserted into the free end of the calcium chlorid tube (*d*), and the pressure of the column allowed to force the liquid to the inner end of the long tube. This forces the gas into the end of the bottle farthest from the flower and allows a gradual distribution by diffusion. For ethylene and illuminating gas water was always used as the forcing liquid.

In determining the toxic limits of illuminating gas and ethylene, 20-liter carboys were used; while smaller bottles were employed in some of the earlier experiments with these gases, as well as with all the determinations of the least toxic gases. The question of the effect of corking a bud or flower in a closed chamber of this kind naturally arises, and suggests a criticism upon the method. It was found that flowers opened without any apparent injury when corked in flasks of only one liter capacity. In all checks and in all cases where the concentration of the injurious gas was below the toxic limit, the flowers bloomed normally while yet in the bottles. To avoid undue rise of temperature within the chambers basket-covered carboys were used. The experiments were carried on in the laboratory greenhouse during the months of May to September. The temperature in the experiments reported varied from 20°–28°, and within this range no noticeable variation in toxicity appeared.

Two varieties of carnations were used—the Boston Market and the pink Lawson. The two varieties vary so little in their sensitiveness and reaction to ethylene and illuminating gas that a description of the responses of one applies equally well to the other.

To make sure that the effect produced by ethylene was not due to some impurity contained by it, parallel experiments were run with ethylene derived by two different methods: (1) by heating concentrated sulfuric acid with absolute alcohol, and (2) by dropping absolute alcohol upon phosphorous pentoxid heated to 200° C. and later raised to 240° C. The ethylene derived from sulfuric acid was washed by the ordinary gas burette and pipette, as described by HEMPEL (**15**: 34–95); first in concentrated sulfuric acid (sp. gr. 1.84) to remove the aldehyde, and later in 33 per cent. potassium hydrate to remove the sulfur dioxid. In each case the washing was continued until no further absorption occurred. The ethylene derived from phosphorous pentoxid was washed similarly, and in addition in copper sulfate (sulfuric acid solution described by HEMPEL, p. 316) for absorption of phosphene, if any should be present. Various samples of the ethylene derived in this way were analyzed. Bromin and fuming sulfuric acid absorbed 96–98 per cent. The unabsorbed portion proved to be air, coming from the generator chamber. The gases thus derived were diluted with air to form mixtures containing 2 per cent. ethylene. The toxicity of the two mixtures was equal.

In discussing the composition of illuminating gas we can hardly do better than quote a paragraph from SMITH'S (**16**) *General chemistry for colleges:*

> The illuminating gas in Europe, and in many of the smaller cities of the United States, is usually coal gas; while in the larger cities of America it is almost always made from water gas. Coal gas is obtained by the destructive distillation of soft coal, and is freed from ammonia and tar by washing and cooling, and from hydrogen sulfid and carbon dioxid by passage through layers of slaked lime. The water gas, made by the action of steam upon anthracite or coke, being composed of carbon monoxid and hydrogen, has no illuminating power. It is therefore "carburetted," that is, mixed with hvdrocarbons, by passage through a cylindrical structure filled with white-hot firebrick, upon which falls a small stream of high-boiling petroleum. The relatively involatile hydrocarbons of which the oil consists are thus decomposed ("cracked"), and gaseous substances of high illuminating power are produced. The following table shows the composition of each of these kinds of gas, together with that of oil gas (Pintsch's) which is composed entirely of the products from "cracking" oil:

Components	Coal gas	Water gas	Oil gas
Illuminants	5.0	16.6	45.0
Heating gases:			
Methane	34.5	19.8	35.8
Hydrogen	49.0	32.1	14.6
Carbon monoxid	7.2	26.1	....
Impurities:			
Nitrogen	3.2	2.4	1.1
Carbon dioxid	1.1	3.0	....
Candle power	17.5	25.0	65.0

These are average numbers, and considerable variations from these proportions are often met with. The illuminants are unsaturated hydrocarbons, such as ethylene and acetylene, and the value of the gas for illuminating purposes depends on the amount of these particular components.

The illuminating gas used in our experiments was water gas of the People's Gas Light and Coke Company, drawn from a tap in the Botanical Laboratory. In numerous analyses of samples of this gas (see HEMPEL, p. 282) absolute alcohol absorbed 0.2–0.6 per cent., and fuming sulfuric acid 11–14 per cent. Absolute alcohol absorbs the so-called hydrocarbon vapors (mostly benzene); and fuming sulfuric acid the heavy hydrocarbons, including acetylene, ethylene, and their higher homologues. Bromin is often used as an absorbent of ethylene. Besides ethylene, however, it absorbs several other constituents of illuminating gas. In a number of analyses this reagent absorbed 9–13 per cent. A more definite determination of ethylene will be given in the experimental portion.

At first the illuminating gas used was washed through 33 per cent. potassium hydrate to absorb any traces of sulfur dioxid and hydrogen sulfid it might contain. This was found not to modify the toxicity, and hence the unwashed gas was used thereafter. The methods of deriving and purifying the other constituents (of illuminating gas) worked with will be described in the later paper, which gives their effects.

3. Experimental

ILLUMINATING GAS

As a later paper will deal fully with the effects and toxic limits of the constituents other than ethylene, we need make only a general statement concerning them here. A number of experiments were run to determine the toxic limits of methane, carbon monoxid, acetylene, hydrogen, carbon bisulfid, and benzene to the buds and flowers. As would be expected, hydrogen was perfectly neutral when it completely displaced the nitrogen of the air. In all the other constituents here mentioned, the toxicity was such that in the least amount of illuminating gas necessary to kill the bud no one is concentrated enough to reach $\frac{1}{50}$ of its toxic limit. It is very probable, therefore, that these constituents play no part in determining the toxic limit of illuminating gas. It has already been stated that the absorption of hydrogen sulfid and sulfur dioxid does not modify the toxicity of the gas. This leaves, then, ethylene, the higher homologues of ethylene and acetylene, and certain aromatic sulfur compounds to account for the toxicity of the gas. All these substances except ethylene exist in very small percentages in illuminating gas. All evidence in the following experiments also points to the conclusion that there is enough ethylene in the gas to account for its toxicity.

The small greenhouse in which entire potted plants were exposed to the action of gas had a capacity of $1.69^{\mathrm{cu.\,m}}$. In order to make comparisons easy between buds of the same size on the plants exposed and on the checks, corresponding buds were tagged with the same numbers. We need describe only one of these experiments. Potted plants of the Boston Market were put into the small greenhouse in the evening and 2 liters of gas were run in at the end opposite the plants, allowing a gradual distribution by diffusion. The plants were taken out the next morning to prevent injury by high temperature. The following evening the plants were returned to the enclosure and left for 60 hours (the following two days being cloudy). At the time they were put in, 4 liters of gas were run in, and the same amount was added 48 hours later, there being at that time no perceptible smell of gas in the chamber. This experiment served to show (1) that the vegetation is far less sensitive to gas injury than the buds, for there was no apparent injury to the vegetation; (2) plants remained vigorous, put out new buds, and later produced other flowers. The oldest buds (those showing color and just ready to open) and the youngest buds (those less than 0.6^{cm} in diameter) were the ones most injured. Many of the medium-sized buds, however, escaped death, although retarded considerably in their growth. The older buds showed a slight growth of the petals, but never opened. Later they shriveled and turned yellow.

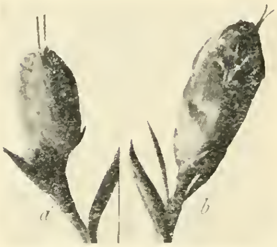

FIG. 2.—*a*, result of treating a bud, just beginning to show the petals, for three days with 1 part of illuminating gas in 20,000; *b*, result of the treatment of a similar bud, for the same length of time, with 1 part of ethylene in 500,000.

Our experiments in which individual buds were enclosed and exposed to illuminating gas began with liter flasks in which as much as 25^{cc} of gas was used. The time of exposure was usually three days, starting when the petals were just beginning to show. A gradual reduction of the concentration by reducing the amount of gas used and by increasing the size of the enclosure finally located the toxic limit. The highest concentration did no apparent injury to the vegetation; but the effect upon the buds was made apparent by a failure to open, by a discoloration and withering of the petals, and by the projection of the stigmas. When using 1^{cc} of illuminating gas to $20,000^{cc}$, the stigmas still project as shown in *fig. 2, a;* 0.5^{cc} of illuminating gas did not sufficiently retard the growth of the petals to cause projection of the stigmas, yet the buds never opened farther than shown in *fig. 3*, although the petals remained fresh for several days. Very young buds were also exposed to the last concentration of the gas (1 part in 40,000, or 0.0025 per cent.) for a period of three days. The injury was not apparent at first, and the buds remained green for several days, but finally turned brown and withered.

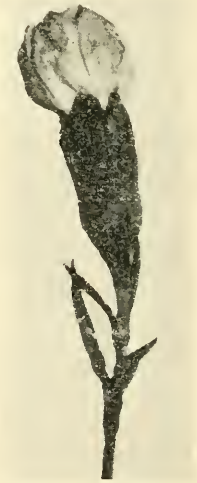

FIG. 3.—Result of treating a bud, just beginning to show the petals, for three days with 1 part of ethylene in 1,000,000.

A series of exposures was also made on the open flowers. We selected for this work those that had just opened, in order to be sure that any change produced was due to the toxicity of the gas rather than to the natural death of the flower. Here as well as in all the other experiments checks were kept. *Fig. 4, a* shows a flower before being corked in a 20-liter carboy; *b*, the same after being corked in a 20-liter carboy (containing air only) for 24 hours; *c*, a flower before being corked in a 20-liter carboy; and *d*, the same after being corked in 12 hours with 0.5^{cc} of illuminating gas. This shows that 0.5^{cc} of illuminating gas per 20,000 (1 part in 40,000) causes the complete closing of the flower in 12 hours or less. Higher concentrations caused a more rapid closing and a marked inrolling of the petals. With 0.5^{cc} per 20,000 and less the inrolling is not conspicuous. Even 0.2^{cc} per 20,000 causes considerable closing in 12 hours, though not as marked as 0.5^{cc}.

The effect of duration of exposure was also tested. No injury was done to a bud just ready to open upon one day's exposure to 2^{cc} of gas per 20,000 (four times killing concentration for three days' exposure). On a similar bud 5^{cc} for one day was considerably more injurious than 0.5^{cc} for three days. The stigmas did not project, but the petals were markedly discolored.

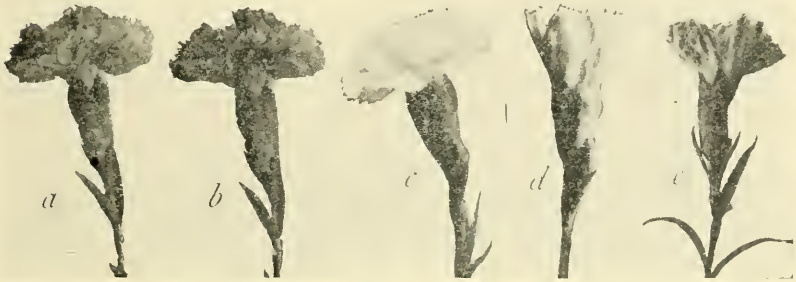

FIG. 4.—*a*, a flower that has just opened; *b*, the same after being corked in a 20-liter flask of air for 24 hours; *c*, a flower that has just opened; *d*, the same after being exposed 12 hours to 1 part of illuminating gas in 40,000; *e*, result of treating a flower that just opened for 12 hours with 1 part of ethylene in 2,000,000.

During the entire period of experimentation there was no very marked variation in the toxicity of the gas used.[1]

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[1] In determining the toxic limits we located a concentration that produced the effect while one-half that concentration did not. It is clear that this permits considerable variation without detection. It is not possible to locate the toxic limits more closely, due to the variation in the flowers. It is clear, however, that this gives a very good idea of the magnitude of toxicity.

ETHYLENE

The experiments with ethylene were begun by exposing buds just beginning to show the petals to 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, and $\frac{1}{16}{}^{cc}$ of ethylene in 20 liters.

In each of these concentrations the buds were killed on three days' exposure. The usual signs of gas poisoning were noted; petals turned yellow and withered, and the stigmas projected. Since it was evident that these concentrations were far above the toxic limit, we resorted to the use of a 2 per cent. mixture of ethylene with air. Various amounts of this were used, until the toxic limits were definitely located. With 2^{cc} of this 2 per cent. mixture in 20,000 (1 part in 500,000), the results were similar to that obtaining with 1^{cc} of gas per 20,000 (1 part in 20,000). In *fig. 2, b* is a bud just showing the petals exposed to this concentration of ethylene for three days. Also 1^{cc} of 2 per cent. ethylene per 20,000 (1 part in 1,000,000) gives results similar to that shown by 0.5^{cc} of illuminating gas per 20,000 (1 part in 40,000). *Fig. 3* shows the results of such an exposure for three days on a bud just showing the petals. The growth of the petals is not sufficiently retarded to make the stigmas conspicuous; the petals remain fresh for several days but never open farther. Where much less than 1^{cc} of 2 per cent. ethylene per 20,000 was used with similar buds, three days' exposure did not prevent their opening.

When open flowers were exposed to the ethylene, it was found that 0.5^{cc} of the 2 per cent. mixture in 20,000 (i e., 1 part in 2,000,000) caused the closing within twelve hours. The result of such an experiment is shown in *fig. 4, e.*

It is seen from the data given above that ethylene must form approximately 4 per cent. of illuminating gas to be the constituent that determines the toxicity of the latter. It becomes necessary now to get an estimate of the fraction of the illuminating gas used that is ethylene. We have already stated that no absorbent used in gas analysis absorbs ethylene alone. In a special absorption chamber, packed in ice, 50 or more grams of bromin with 150^{cc} of water were placed, and measured quantities of illuminating gas passed through. When the bromin water was partially discolored, showing an almost complete exhaustion of the bromin, the resulting oil (a mixture of ethylene dibromid and other compounds resulting from the reaction of the gas constituents with bromin) was separated, washed with a weak solution of potassium hydrate, and later with distilled water. This oil was then dried with fused calcium chlorid and later fractionated. In the first distillation all the portion boiling between 129° and 134° C. was saved. This was later redistilled and the fraction boiling between 103° and 132° C. saved as representing the ethylene dibromid, since this compound boils at 131° C. About 3 per cent. of the dried material absorbed by bromin boiled between 130° and 132° C.; a small portion boiled at 129° C. or below. From this it rose up quickly to 131° C., where it again gave a considerable fraction. Then it rose rapidly to 139° C., where a considerable fraction distilled. In one trial, 208 liters of gas at 27° C. and under pressure of 745.5^{mm} of mercury gave 130^{gm} of dried oil; of this 44.2^{gm} boiled between 130° and 132° C. After correcting for pressure and temperature the following equation equals the percentage volume of gas that is ethylene:

$$\frac{22.4\times760\times44.2\times300}{208\times745.5\times178\times273}=2.9+\text{ per cent.}$$

In a second determination 138 liters of gas at 27° C. and 745.5^{mm} pressure gave 31.6^{gm} of oil boiling between 130° and 132°. Correcting for temperature and pressure, the following equation gives the percentage volume of ethylene in this case:

$$\frac{22.4\times760\times31.6\times300}{138\times745.4\times178\times273}=3.2\text{ per cent.}$$

It must be stated, however, that according to WINKLER (**17**) the absorption of ethylene by bromin is not complete, and farther that considerable ethylene dibromid is necessarily lost in washing, drying, and distilling, so that the percentage is probably considerably higher than here obtained. It must be urged also that the presence of other oils with boiling points rather near that of the ethylene bromid tends to make this fractionation less accurate.

4. General

It is of great interest to know that the most delicate chemical test for illuminating gas in the atmosphere falls far short of detecting amounts that work havoc with the flowers of the carnation. The tests for carbon monoxid are those used for detecting illuminating gas. The most delicate application of the blood test (see Hempel, p. 225) will detect 1 part of carbon monoxid per 40,000. The iodine pentoxid test (see Hempel, p. 226) is of equal delicacy. If carbon monoxid forms 25 per cent. of illuminating gas, these tests will detect 1 part of illuminating gas in 10,000. Upon three days' exposure 1 part of illuminating gas in 40,000 kills the young buds and the petals of the flowers just beginning to open; while 1 part in 80,000 causes open flowers to close upon an exposure of twelve hours.

The so-called "sleep" or closing of the carnation is a source of considerable loss to growers and dealers, for flowers that once close never again open. This "sleep" is especially likely to occur with cut flowers brought into city markets. Some varieties are so disposed to react in this way that their cultivation has almost entirely ceased. We know several homes lighted with gas where cut carnations can be kept only a few hours without "going to sleep." In one instance the displacement of gas lights by electric lights entirely overcame this difficulty. Our experiments show clearly that one cause of this sleep is traces of illuminating gas (ethylene) in the surrounding atmosphere.

Stone (**13**), Wehmer (**8**), and others have shown that illuminating gas diffuses great distances through the soil, especially if there is a hard-packed or frozen crust over the top. This paper shows the extreme sensitiveness of the carnation to this substance. From these facts it is evident that carnation growers whose greenhouses are in the region of gas pipes must take great precautions against losses from this source. It would be interesting to know whether solid cement walls set into the ground for some depth on the side next the pipes would furnish sufficient protection against leaks of this kind. It is clear that, if (as our results seem to indicate) the group of illuminants, or more accurately if one constituent of this group (ethylene) determines the toxicity of illuminating gas, coal gas is considerably less toxic than water gas, while oil gas is more toxic than either of the others; also the toxicity reported by the German investigators who used coal gas is less than that shown by the gas of the great American cities.

While it seems probable that the limit of toxicity of illuminating gas on the flower of the carnation is determined by the ethylene it contains, it does not follow that such is the case with all parts of plants or even with the flowers of all plants. It would be interesting to know the effects and toxic limits of illuminating gas and its constituents upon various double as well as single flowers. Similar data for the foliage of various plants such as Coleus, which is supposed to be especially sensitive to illuminating gas, would likewise be of interest.

5. Summary

1. The flowers of the carnation are extremely sensitive to traces of illuminating gas in the air.
2. With the Boston Market and pink Lawson three days' exposure to 1 part in 40,000 kills the young buds and prevents the opening of those already showing the petals. The buds of medium age are considerably more resistant.
3. In the same varieties 1 part in 80,000 causes the closing of the open flowers upon twelve hours' exposure.
4. This injury takes place directly on the bud or flower exposed and not indirectly through absorption by the roots.
5. No chemical test is delicate enough to detect the least trace of illuminating gas that will cause serious injury to carnations.
6. The "sleep" of the carnation is probably often caused by traces of illuminating gas in the air.
7. Ethylene is even more fatal to the flowers of the carnation.
8. Three days' exposure to 1 part in 1,000,000 prevents the opening of buds just showing the petals.
9. Twelve hours' exposure to 1 part in 2,000,000 causes the closing of flowers already open.
10. There is much evidence that indicates that the toxic limit of illuminating gas upon these flowers is determined by the ethylene it contains.

The University of Chicago

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FLORAL SUCCESSION IN THE PRAIRIE-GRASS FORMATION OF SOUTHEASTERN SOUTH DAKOTA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 117

(WITH FOUR FIGURES)

LeROY HARRIS HARVEY

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AUGUST 1908

FLORAL SUCCESSION IN THE PRAIRIE-GRASS FORMATION OF SOUTHEASTERN SOUTH DAKOTA

THE PREVERNAL, VERNAL, AND ESTIVAL ASPECTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 113

LeRoy Harris Harvey

(WITH THREE FIGURES)

The western part of Iowa, the eastern and northeastern counties of Nebraska, and southeastern South Dakota lie in the drainage basin of the Missouri. This tri-state area is well within the prairie region of that vast and far-reaching prairie province of the middle west. This area is only a part of the Ponca District of Pound and Clements,[1] being more strictly Dakotan than Nebraskan, and may be considered as representing a transition between the more mesophytic eastern areas of Iowa and those dominantly xerophytic somewhat to the west, with which it shows the closer floristic agreement. Its composition is thus twofold, pointing to the primitive and more xerophytic stages of the past and at the same time prophetic of the mesophytic stages to come. This aberrant character links it strongly to the xerophytic prairie to the west and southwest, from which it is genetically descended, and the prophetic character links it to the more mesophytic prairie of western Iowa, which has encroached ever westward. Under this migration tension from the southeast and east the primitive prairie has retreated, civilization always being a potent factor in this succession.

Study began in this tri-state region in the fall of 1903 at Sioux City, Iowa, and was carried on during that fall and the next summer

JUN 13 1921

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[1] Pound, R., and Clements, F. E., Phytogeography of Nebraska. 1900.

in the contiguous portions of Iowa, Nebraska, and South Dakota. In the fall of 1904, Yankton, South Dakota, became the base station, and for the next three seasons (1904–1907) the work was carried on from that point. During this period the greater part of South Dakota east of the Missouri was visited, and repeated trips were made into contiguous Nebraska and into Iowa, while at the same time detailed study of a restricted area was being prosecuted. This local area upon which study has been focused, lies in the township of Yankton and embraces about 49 hectares, being a rectangular strip 1210^m north and south by 405^m east and west. The topography is uneven and comprises a series of prairie knolls and slopes, separated into two groups by a gentle drainage valley which traverses it in a southwest and northeast direction. The knolls are low and their slopes gentle, scarcely higher than 10 or 15^m, with a gradient never exceeding 30 or 40°. The entire area is largely underlaid by glacial gravel and clayey till. The former mainly constitutes the knolls —the humus is here the most shallow, averaging only about 15 to 20^{cm} in depth. Off from the knolls on the level the humus caps the deposit of clayey till. On the lower erosion slopes (25 to 30^{cm}) and in the drainage valley (60^{cm} plus) the humus has accumulated to a greater depth, sufficient in the latter to bury the till beyond the zone of root activity. The humus is in all cases till or gravel modified by atmospheric, organic, and biotic agencies.

To insure uniformity the nomenclature of BRITTON and BROWN'S *Illustrated flora* has been followed, except where it has conflicted with the verification of grasses made for me through the United States Department of Agriculture by MR. PERCY L. RICKER and MR. D. A. BRODIE, to whom I am greatly indebted for this courtesy.

This problem has been carried on under the direction of DR. HENRY C. COWLES, to whom I wish to acknowledge my indebtedness for many valuable suggestions and criticisms during the progress of the investigation.

Geology and topography

To appreciate fully existing conditions, an epitome of the post-Cretaceous geological development of the region is necessary. The Cretaceous was terminated by that great uplift and crustal movement which formed the Rocky Mountains and gave birth to the major features of the Missouri valley drainage. This movement particularly affected this region by elevating it from an estuary condition to a point considerably above sea-level, and even probably many feet above its present level. This uplift inaugurated a period of vast erosion, and before the advent of the ice the Missouri had cut its present great valley at least 20 to 25^{m} below its present level.

With the Pleistocene came the glaciers. While doubtless there were five periods of glacial advance and recession in the region, we need concern ourselves now only with the second, the Kansan, which spread from the Keewatin center and deposited over this entire region the Kansan drift sheet, obliterating the questionable pre-Kansan. The Illinois, Iowan, and Wisconsin epochs followed successively, only the latter reaching into southern South Dakota, where a lobe of the Altamont moraine pushed down between the Big Sioux and Missouri rivers, reaching approximately to Vermillion, South Dakota.

The Kansan must have seriously interfered with the established preglacial drainage, greatly rejuvenating it, at least along minor lines. It seems probable also that subsequent erosion mainly sought out previous lines, largely reestablishing the post-Pliocene drainage system. The Wisconsin likewise disturbed and caused a readjustment of this drainage system, which could have differed but little from that previously worked out in the Kansan. Upon this readjustment of the post-glacial Wisconsin drainage topography, there followed the deposition of that much mooted deposit, the loess.

The region divides itself naturally into two great topographic types, the rolling upland prairie and the flood plain, which cuts the prairie in a general northwest and southeast direction. On either side the flood plain is limited by the escarpments of the Missouri. From this flood plain extended the minor flood plains of its tributaries, further dissecting the upland. The vast valley is cut out from 25 to 50^{m} deep in the upland and presents a flood plain varying from a narrow terrace to frequently 10^{km} in width.

The upland now presents an almost perfectly developed erosion topography, predetermined in the Kansas drift sheet and subsequently veneered by the Wisconsin drift and loess deposit. The latter, which frequently has a depth of 50^{m} to the south of the Wisconsin drift area, thins out northward. The escarpment bluffs are steep and high, but pass back into a complex of gently rounded, semi-detached hills, which present a sinuate or undulating sky line of jumbled peaks; it is a vast mountain system in miniature. Back a few miles from the bluffs these hills pass imperceptibly into the low, rolling prairie hills, which extend with scarcely a variation in either direction. So perfectly is this tributary drainage system established that upon the prairie, swamps and "sloughs" rarely occur. The small streams which have threaded the upland are characterized by ravines of depth and precipitousness, especially in the loess, where they usually end abruptly in a bluff, again dividing the upland into a series of ridges, intricately related, which pass back into the low, rolling hills of gentle profile.

Origin of the prairie

The uplift of the Rocky Mountains, which terminated the Cretaceous, introduced a modifying element which exercised an ever-increasing influence upon the climate of the Great Plains region. Intercepting the eastward-moving moisture-laden winds from the Pacific, a decrease in the annual precipitation to the east of the range must of necessity have followed. The greatest reduction would have been nearest the mountains, decreasing to the eastward. When this interior continental land was finally left by the interior sea and opened to migration, invasion must have been in large degree controlled by this graduated distribution of rainfall. The subsequent origin of the Cascades could have served only to accentuate this distributional difference and reduction in precipitation. Under such conditions the Tertiary phytogeographical distribution of this central region must have been adjusted. Whatever the source, it would seem highly probable that while the entire Mississippi valley was occupied by the rich Tertiary forest, the region lying to the west and bounded by the Rocky Mountains and extending northward into Assinaboia was, on account of the low precipitation, denied to tree invasion and came to be occupied by a prairie formation, increasing in its xerophytism westward just as it does today.

Toward the close of the Tertiary (late Pliocene) fossil evidence points conclusively to climatic change. A retrogressive succession of floral waves swept southward under the influence of the gradually falling temperature of the Pleistocene. In this glacial movement, the plains region, unable to support tree growth, acted as an entering wedge, causing an east and west divergence. One wing of the migration, dominantly coniferous, followed the Rocky Mountains southward; the other, typically deciduous, sought the Mississippi and its tributary valleys as a migration track; while the prairie moved directly toward the Gulf. At the time of maximum ice advance the descendants of the Tertiary forest were mobilized in the southern Appalachians about the Chattanooga region as a center (ADAMS **:02**), while the prairie formation concentrated in the southwestern United States, with a possible center in the region of northeastern Texas and eastern Oklahoma and southern Kansas.

With final glacial retreat from this region and subsidence of the glacial sea, migration tension was removed and distribution tension became active. The life waves now in succession rolled northward. The content of our flora demands a consideration of the third wave only. A study of the floristics shows indisputably the commingling of forms of diverse geographical affinity. An unmistakable floristic relation, in many cases specific, exists with a southwestern and southeastern center of post-glacial dispersal.[2] To the east and southeast the deciduous forest type becomes increasingly characteristic, while to the west and southwest the plain or prairie type gradually predominates; the region thus lies in the western border of the tension zone in which migration from these two competing centers of distribution meet. From the southeast the dispersal route has been up the Missouri valley; while the northwestern migration has spread diagonally across natural drainage lines, following the upland plains.

Forest invasion of the prairie

When the arborescent elements of the southeastern biota, migrating up the valleys of the Mississippi and of the Missouri and its tributaries, reached the prairie region they were unable to occupy the high lands, but occupied the flood plains and adjacent slopes. There is no evidence to indicate that the then existing topographical and climatic conditions differed radically from those of today. Again these elements have today their most widespread distribution, a condition at once intelligible when related to physiographic development in the working-back of streams and the increase of flood plain. Two salient points may now be noted: the initial and the continuous subsequent preemption of the upland by the prairie formation, and secondly the continuation of initial climatic conditions. The initial causes which operated to restrict tree invasion and ecesis upon these prairie-covered uplands would thus seem to have their duplication in those natural factors which operate to that end at the present time.

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[2] BESSY (**'99**, p. 82) says: "There are 66 or 67 species of native trees in Nebraska, and 56 or 57 have advanced to the state from the southeast." MACMILLAN (**'92**, pp. 653, 721) has shown that 66 per cent. of genera indigenous to the Minnesota valley are eastern and 62 per cent. southern, while 32.5 per cent. of the total species are of southeastern origin. Of the native trees of South Dakota at least 75 per cent. show indubitable southeastern affinities. The presence of such genera as Opuntia, Cactus, Yucca, Mentzelia, Croton, Bouteloua, Bulbilis, Lygodesmia, and Aplopappus strongly bespeaks the southwestern alliance.

Under the discussion of ecological factors of the region it will be shown that there is a coincidence of factors operating most antagonistically against tree growth upon the upland. The almost entire absence of fungi upon the prairie cannot but be significant. The roots of most of these deciduous trees are obligatively provided with symbiotic mycorhiza and the absence of their specific fungus would preclude advance. Hence fungal infection of the prairie soil must precede or at least accompany forest encroachment. Again, the difficulty of seed germination, almost impossible either because of a dense sod or a lack of soil moisture, successfully checks invasion. In consideration of these precarious climatic factors, peculiar edaphic conditions, and the fact that if planted upon the prairie trees thrive, I am led to the view that the question of non-invasion upon the prairie proper is primarily and initially one of pre-occupation and the inability of seedlings successfully to withstand the extremely severe conditions of the first winter's exposure. In the positive and coincident interaction of unfavorable biological and climatic conditions may be found a cause sufficient to account for tree absence upon the prairie and the slow migration of tree species into this region.

While these causes are all sufficient, yet we must not disregard a secondary and artificial though highly cooperative factor, which in recent times must have served in many places to prevent tree establishment. I refer to prairie fires; yet even in the absence of prairie fires for half a century the prairie stands uninvaded except in cases of physiographic development (Bessey '99). And again, as the prairie existed as a climatic formation long before these fires, either of Indian or Caucasian origin, swept the prairies, it would appear that this fire factor has been overestimated by many and is in no sense to be regarded as the fundamental factor. That there is, nevertheless, an extremely slow advance of the forest usually through the medium of its forerunner, the shrub association, whose pioneer *Symphoricarpus occidentalis* is followed by *Rhus glabra*, is very evident.

While the above factors are seemingly adequate to account for tree absence upon the western upland prairie, yet in light of the prairie as a natural climatic formation it would seem more proper to make the problem one accounting for the occasional presence rather than the general absence of trees. A future paper is planned to discuss the factors controlling this encroachment.

The period of growth resumption

Not until about the first week in March are climatological conditions at all favorable to an awakening of vegetation, and then only on infrequent days; but the month as a whole is marked by the opening of flower and leaf buds of trees and the beginning of the germination of prevernal annuals and the formation of the basal rosettes of the perennials, though some tide over from the previous season. The prevernal bloomers naturally make most rapid progress, aided by their geophytic habit. Not infrequently the temperature falls below freezing and killing frosts ordinarily result. Light snow storms, which rarely occur, may temporarily retard growth. The conditions become progressively more favorable and pass insensibly into the period of the prevernal flowers.

The usual snows and rains of the early part of the month assure abundant moisture. The chresard, which is about 18 to 20 per cent. in the early days of the month, decreases to about 14 per cent. at the middle, and to about 12 per cent. toward the last of the month, thus giving an average chresard of some 15 per cent. The different exposures of the prairie hills, knolls, and ravines progressively recover from the effects of winter in the following order: southeast to southwest, southwest to northwest, northeast to southeast, and northwest to northeast. The frost clings to the northern exposure in ravines, only leaving toward the latter part of the month, particularly when this exposure is forested. On March 16 on a northwest exposure of a prairie knoll the frost was recorded at the following depths: at base of slope 13.97^{cm}, at middle of slope 30.48^{cm}, at crest considerably below 35^{cm}. Thus the base of the exposure recovers much more slowly than the crest, a condition holding equally true for all exposures; hence vegetation starts earlier upon the upper slopes. However, the chresard increases toward the base of the slope. On March 16 the slope in question showed a chresard at the crest of 7.1 per cent., at the middle of 14.1 per cent., and at the base of 18.2 per cent., the differences being more pronounced and the chresard greater than in any subsequent aspect.

The physical conditions of the period are shown in the adjacent table.

TEMPERATURE*			WIND*			RAIN*			LIGHT*			SOILS§			MEAN RELATIVE HUMIDITY†	RELATIVE EVAPORATION‡
Maximum	Minimum	Mean	Prevailing direction	Total movement	Hourly velocity	Days	Mean daily	Total	Relative¶ intensity	Mean cloudiness	Sunshine per cent.	Mean holard	Mean echard**	Mean chresard		
22 C.	−17 C.	2 C	N.W.	7666	10.3	8	$.11^{cm}$	3.35^{cm}	.459	5.2	51	21.3%	7.2%	14.7%	75.2	.510

* Data from Yankton, United States Weather Bureau, being the means of ten years (1894–1904). The nearness of the station to the field makes the data very representative.

† Data from Sioux City, United States Weather Bureau, being the mean of 8 A. M. and 8 P. M. observations from July 1, 1889, to December 31, 1903. The station is about 30 miles south of Yankton.

‡ Relative evaporation is determined by the formula: R.E. = saturation deficit × average hourly wind velocity × mean temperature centigrade ÷ 1000. It is recognized that such a result is by no means quantitative, yet it may well serve relatively as an index, invaluable in contrasting the floral aspects.

§ Soil data all from station B, Solidago slope, N. W. exposure. For technique see CLEMENTS, Research methods in Ecology. 1905.

¶ Relative light intensity equals sunshine per cent. of theoretical maximum light intensity as determined for Yankton by method of sun's altitude.

** Determined from an experiment on the Windsor bean. It will be used as an index of the non-available water. The chresard is then directly computed from the holard.

Prevernal floral aspect

Floral activity begins only in the first week of April, and is characterized by the slow and progressive flowering of six forms, extending up to the last week in April, when an apparent break in floral continuity occurs, no forms flowering for a week or ten days. This break very naturally segregates the prevernal floral aspect.

In addition to the floral forms this aspect is conspicuous as the time of appearance of the rosettes of the vernal, serotinal, and autumnal perennials, and seedling annuals of these same aspects. No facies is established, and the tone of the formation is that of winter and early spring, mainly produced by the standing brown stalks of *Solidago rigida*, *Helianthus scaberrimus*, and *Verbena stricta* of the autumnal aspect, which gives everything a brownish cast, enlivened here and there by the mats of *Antennaria campestris* and *A. neodioica*, and at the base of slopes and in depressions by the green of *Poa pratensis* sod. At the base of slopes now and then are to be seen clumps of green produced by the unfolding leaves of colonies of *Symphoricarpus occidentalis*. To complete the aspect, dotted here and there are the floral forms which characterize it. Ruderal species are noticeably absent.

The pertinent climatological conditions of the prevernal floral aspect may be obtained directly from the adjacent table. On only 63 per cent. of the days does the mean average temperature rise above 6° C., with a range between −6° and 29° C. Coupling this with an average soil temperature of 13° C., growth conditions are seen to be far from favorable. The prevailing wind direction is northwest and with a mean

PREVERNAL CLIMATOLOGY

Temperature			Wind			Rain			Light			Soil			Mean relative humidity	Relative evaporation
Maximum	Minimum	Mean	Prevailing direction	Total movement	Hourly velocity	Days	Mean daily	Total	Relative intensity	Mean cloudiness	Sunshine per cent.	Mean holard	Mean echard	Mean chresard		
29° C.	−6° C.	11° C.	N. W.	7919	11	10	.26^{cm}	7.87^{cm}	.547	4.7	63	20%	7.2%	12.8%	63.7	4.392

hourly velocity of 11 miles, which is not exceeded during the other aspects. Precipitation is slight (7.87^{cm}) and falls on only about 33 per cent. of the days. The average cloudiness of the sky (4.7) is low, and the sunshine (63 per cent.) is correspondingly higher, resulting in a low light intensity (0.547). The saturation deficit is here not at its maximum (36.3), but, augmented by the high wind velocity, evaporation, which must necessarily serve as a rough comparative index of transpiration, is rising in amount. While far more favorable for vegetation, it becomes progressively more so, the last half of the aspect presenting conditions in soil and air noticeably more congenial to growth. The chresard decreases steadily during the aspect from 14.8 per cent. on April 17 to 12.2 per cent. on April 25, and a marked difference in holard was evident at crest (15.8 per cent.), slope (18.4 per cent.), and base (22.6 per cent.) on April 18. The average chresard is 12.8 per cent.

SPECIES OF THE PREVERNAL ASPECT

FACIES.—None.

PRINCIPAL SPECIES.—Antennaria campestris,† Carex pennsylvanica,† Peucedanum nudicaule,† Pulsatilla hirsutissima.*†

SECONDARY SPECIES.—Astragalus crassicarpus,† Peucedanum foeniculaceum,* Draba micrantha, Ranunculus ovalis.

* Not occurring in area proper but in vicinity. † Forming associations.

The earliest flowering form is Pulsatilla and occurs copiously to subcopiously and characteristically upon the upper slopes of the prairie hills. It appears several days earlier upon the south to southwest exposure, which holds equally true for the other prevernal bloomers. The early warming-up of this exposure accounts for the above phenological precocity. But the greatest abundance of these prevernal forms occurs on the north to northeast exposure. Maturation follows close upon anthesis, which likewise holds for all prevernal flowering species.

Pulsatilla is followed by the blooming, during the first week of April, of *Peucedanum foeniculaceum* and *P. nudicaule.* The former with its umbel of yellow flowers is of rare occurrence; but the latter with its umbel of white flowers appears even copiously in restricted plats, and in its distribution occurs mainly upon the upper xerophytic slopes of prairie knolls where the grasses are bunched and the association more or less open, though rarely seen at the crests; it thus not infrequently exerts a subtone effect at short range. It is perennial by means of its geophytic root. With the elongation of its peduncle and spreading of its umbel in maturation during the last week of the aspect, it becomes more conspicuous and persists thus far into the vernal aspect.

The beginning of the second week of the prevernal aspect sees *Carex pennsylvanica* in full bloom. It occurs copiously, extending well up to the crests of knolls, but more abundantly on lower slopes, yet never influences the tone of the aspect. In places Carex may assume almost facial rank, but always maintains a bunchy or isolated distribution, which, however, is quite general. The peculiar yellowish-green shade of its leaves and its yellow staminate spikes which appear after the stigmas make it conspicuous. It is a perennial of xerophytic tendencies, propagating itself by rootstocks and stolons.

Carex is shortly followed by the flowering of *Antennaria campestris*, and with its white tomentose leaves, scapes, and papillate heads, it gives a characteristic local tone to this floral aspect, even from a distance. It occurs usually gregariously, being one of the two mat-forming species of the formation, and is very generally distributed throughout, facilitated by its perfect adaptation to wind dispersal. The mats themselves may be isolated or gregarious, as many as twenty having been noted in a plat of $64^{\text{sq m}}$, yet single mats frequently cover $16^{\text{sq m}}$, averaging about 1000 plants to the square meter. Propagation is by stolons and migration is centrifugal, with a slow but positive occupation. Mats unite and take complete possession of extensive plats, yet it yields before *Poa pratensis*, in no way being able to hold its own against this sod-forming mesophyte.

The prevernal floral aspect of the formation is terminated by the appearance of *Ranunculus ovalis*, *Astragalus crassicarpus*, and *Draba micrantha* toward the end of the third week of April. They appear in bloom almost simultaneously, but in no way contribute equally to the aspect. Draba occurs only where the soil is exposed and the grass is bunched, hence on upper xerophytic slopes, and appears to be forced out whenever a close association is formed. It is an annual of marked xerophytic tendencies, its leaves being basal and heavily pubescent with stellate hairs. It is scarce and its small white flower inconspicuous, so in no respect does it influence the floral tone. Ranunculus is even more restricted, occurring sparsely on lowest slopes and in depressions on the higher slopes, and is apparently related to a high water content of the soil. Its yellow petals soon fall, and its presence might easily be overlooked in a casual survey of the formation. It is a perennial and is an index of mesophytic conditions. Astragalus, with its racemes of violet-purple flowers, is easily marked in the formation. While generally distributed, its abundance is sparse to subcopious, yet frequently it assumes a gregarious habit. It is a perennial of thickened tap roots which branch above and eventually fragment behind, establishing new individuals. Its migration is slow; dispersal is effected mainly by gophers, which store the fruits for winter consumption. However, ecesis is very certain.

Vernal floral aspect

Toward the last of the first or the beginning of the second week in May there is a floral outburst inaugurated by the blooming of *Nothocalais cuspidata* and *Lithospermum angustifolium*, closely followed by *Castilleja sessiliflora*, *Lithospermum canescens*, *Viola pedatifida*, and *Oxalis violacea*, which marks the inception of the vernal floral aspect. Forms are now progressively added up to about the first week of June, when the aspect is distinctly terminated by the general blooming of certain sod-formers. *Astragalus crassicarpus* and *Ranunculus ovalis* have extended over into this aspect, the former reaching its maximum flowering about the second week in May, thus entering conspicuously into the vernal period. The fruiting scapes of *Peucedanum nudicaule* enter into the tone, while *Antennaria campestris* with its white fruiting heads is now more noticeable than earlier. The deadened brown tone of the prevernal aspect is at last relieved and replaced by the green of the grassy sod, which is rendered somewhat bizarre by the very general distribution of some twenty-eight flowering forms, the largest number occurring in any aspect. No floral facies is developed except in the case of *Poa pratensis* at the base of slopes, and then only in the later part of the aspect. Most of the prairie annuals have by the later part of the aspect appeared and those of the following aspect are now all ready to bloom. Much growth has taken place in the summer perennials and they have apparently outstripped the autumnal forms. Together they overtop the majority of the vernal group, and toward the end of the aspect the large cauline leaves of Solidago and Helianthus of the autumnal aspect render many of the low-statured bloomers quite hidden, except in the very open associations on the highest slopes and crests. Four ruderals bloom in this aspect but have little influence on the formation.

The summary clearly indicates the significant climatological facts. Physiological activity ensues practically throughout the aspect; the temperature range of 31° C. to 2° C., with a mean of 16° C., rarely inhibiting growth. The wind is dominantly from the south and east quarters. It reaches during this aspect a less total movement and so a less mean hourly velocity than in the prevernal, but the atmosphere has a much lower relative humidity; the relative evaporation is thus nearly twice as great. Couple this with the highest light intensity (.704) and the beneficial results of 8.19^{cm} of precipitation on twelve days are much reduced. The chresard shows a marked decrease from 14.1 per cent. on May 10 to 7.4 per cent. on May 22, though the average chresard for the aspect is 10.7 per cent. The base (12.7 per cent.), slope (14.6 per cent.), and crest (8.8 per cent.) on May 22 still showed a gradation in holard, though the distinction of position is less marked than in the prevernal aspect.

VERNAL CLIMATOLOGY

TEMPERATURE			WIND			RAIN			LIGHT			SOIL			MEAN RELATIVE HUMIDITY	RELATIVE EVAPORATION
Maximum	Minimum	Mean	Prevailing direction	Total movement	Hourly velocity	Days	Mean daily	Total	Mean cloudiness	Sunshine per cent.	Relative intensity	Mean holard	Mean echard	Mean chresard		
31 C.	2 C.	16 C.	E.	6877	9.2	12	.26cm	8.19cm	5.2	73	.704	17.9%	7.2%	10.7%	51.7	7.109

SPECIES OF THE VERNAL FLORAL ASPECT

FACIES.—Poa pratensis† (base of slopes and in depressions).

PRINCIPAL SPECIES.—Spiesia Lamberti sericea, Sisyrinchium angustifolium,† Antennaria campestris,*† Spiesia Lamberti, Castilleja sessiliflora.†

SECONDARY SPECIES.—Viola pedatifida,† Oxalis violacea,† Lithospermum canescens, Carex Meadii,† Meriolix serrulata, Hedeoma hispida, Plantago Purshii,† Antennaria neodioica,† Lithospermum angustifolium, Oxalis stricta, Carex festucacea,† Polygala alba, Poa compressa,† Astragalus crassicarpus.*†

TERTIARY SPECIES.—Pentstemon gracilis, Nothocalais cuspidata, Linum rigidum, Lappula texana, Astragalus plattensis, Gaura coccinea, Senecio plattensis, Psoralea esculenta, Vicia linearis, Osmodium molle, Astragalus hypoglottis, Viola cucullata.

RUDERAL SPECIES.—Lepidium virginicum, Melilotus officinalis,† Melilotus alba,† Lappula Lappula.

* From earlier aspect. † Forming associations.

The first week in May is marked by the flowering of *Nothocalais cuspidata* and *Lithospermum angustifolium.* The solitary yellow head of the false calais, frequently 5^{cm} in width terminating a long (20 to 30^{cm}) naked scape, makes it very conspicuous, though it is never more than sparse to subcopious. It is largely confined to loosely sodded ridges and high slopes, and is pronouncedly of xerophytic tendencies. It is perennial by an excessively thickened root. The achenes are heavily provided with pappus, assuring a wide dissemination.

Nothocalais has scarcely bloomed before the puccoons are in flower, *L. angustifolium* appearing several days before the hoary puccoon (*L. canescens*). The former with its terminal leafy racemes of light-yellow flowers and its sparse occurrence remains an inconspicuous element. The hoary puccoon occurs throughout with the other, but more abundantly. Its orange-yellow flowers in a compact leafless umbel make it perhaps the most conspicuous though it is not the most abundant element in the vernal aspect. Both of the puccoons are abundantly pubescent and in their structure and distribution show marked xerophytic tendencies. They are perennials by deep thick roots. The smooth nutlets preclude all but a very limited migration.

Castilleja sessiliflora, *Viola pedatifida*, *Oxalis violacea*, *Sisyrinchium angustifolium*, and *Spiesia Lamberti sericea* bloom progressively during the second week of the aspect, and together produce a very noticeable change in the floral tone, a change which is further accentuated by the forms that occur later. The yellow tone given by the puccoon becomes dotted here and there by yellow, blue, violet, white, and purple, and a bizarre tone is the result. Castilleja occurs rarely and of a very restricted distribution, being confined to associations upon upper slopes, where its pale-yellow flowers render it always inconspicuous. The thickened perennial roots are parasitic upon the roots of other plants. Many flowering stalks may arise from one root, thus assuming a bunch habit. The high immobility of the seeds, resulting in a very restricted distribution, accounts for its gregarious habit and local occurrence upon the prairie.

Viola pedatifida with its bright-blue flowers and unrestricted distribution and sparse to subcopious abundance exerts a characteristic effect on the vernal tone. The prairie violet is a perennial from a fleshy short rootstock.

Oxalis violacea seems somewhat restricted to middle and lower slopes, where it may occur densely in open, matlike patches, resulting from a slow centrifugal migration through bulb formation coupled with a positive ecesis. Though acaulescent and of low stature, its gregarious habit and rose-purple flowers, with their green background of palmately trifoliate leaves, make it in restricted plots of primary floral importance in the aspect. It likewise occurs sparsely but generally distributed over all parts of the formation, with the exception of crests and Poa sod, where it exerts only a minor effect. The shallow scaly brown bulbs indicate its perennial nature. Dissemination is by propulsion, the few seeds formed being not distantly ejected from the pentalocular ovary by the recurving of the loculicidally dehiscing carpels. In this we have a still further explanation of its gregarious tendency.

Characterized by a general distribution, a subcopious abundance, and the "bunch-habit" of growth which aggregates its many blue and white flowers, *Sisyrinchium angustifolium* becomes of first prominence in the vernal floral aspect. It is perennial by short fibrous rootstocks. It is abundantly fertile, and the smooth ovoid seeds are discharged but weakly by the loculicidally dehiscing tricarpellate capsule.

The stemless loco-weeds, *S. Lamberti* and its somewhat earlier flowering and more abundant silky variety, *S. Lamberti sericea*, are acaulescent perennial herbs from thick deep roots. Their dense spikes of dark-purplish flowers borne on long peduncles, a restricted though subcopious distribution upon the higher slopes, and silky pubescence make them very conspicuous, easily dominating in the aspect. The middle of the aspect is controlled by the variety, but the type, which flowers some ten days later, holds the floral dominance for the remainder of the vernal aspect. The type is decidedly more mesophytic in its structure and distribution, and it seems evident that the variety is very possibly a xerophytic mutant of *S. Lamberti.* Propagation occurs through fragmentation of the perennial root, producing as in Astragalus a gregarious habit. A limited seed dispersal furthers this patchy distribution.

Noteworthy on account of their rarity in the aspect are *Astragalus plattensis*, *A. hypoglottis*, and *Viola cucullata*, all flowering toward the end of the second week in May. The Astragali occur only in open association toward the crest and are strikingly inconspicuous. They are both decumbent and perennial. The two-valved pod of *A. plattensis* is fleshy and dehiscent and its seed dispersal is accomplished through limited propulsion. *Viola cucullata* is noteworthy, as only a single individual has been recorded in the area and that on the middle slope of a northwest exposure, doubtless the result of fortuitous distribution.

The third week is characterized by the blooming of *Antennaria neodioica*, *Carex Meadii*, *C. festucacea*, *Vicia lineraris*, *Senecio plattensis*, and *Pentstemon gracilis*. Early in the third week *Antennaria neodioica* flowers; it appears to be more mesophytic than *A. campestris*, occupying the lower slopes, and it occurs less abundantly, but with the fruiting scapes of the earlier species the antennarias are scarcely second to any forms in conspicuousness. *A. neodioica* is a stoloniferous perennial and forms mats, its rosettes living over winter. It is easily distinguished even at a distance by its large and lighter-colored mats, and by the fact that it flowers while the other species is undergoing maturation and distribution.

The carices appear at about the same time toward the last of the week. *C. Meadii*, however, is earlier and occurs sparsely on lower slopes and is found inclusive in Poa sods. *C. festucacea* occurs likewise on lower slopes, but more abundantly and assumes the "bunch-habit" of growth. Both the carices perennate and propagate vegetatively by rhizomes. They contribute little if any to the floral tone of the aspect.

Florally associated with the carices, as just noted, are *Vicia linearis*, *Senecio plattensis*, and *Pentstemon gracilis*, to which are soon added *Lappula texana* and *Plantago Purshii*. Vicia occurs rarely, is confined to lower slopes, and exerts no tone effect; it is a perennial form and is pollinated by bees. Senecio, also of rare occurrence, seems to be confined to mesophytic portions of the formation. It is conspicuous on account of its numerous yellow heads and ray flowers. But for its limited occurrence it might easily dominate the tone, and in the vicinity of Sioux City it was recorded as controlling the aspect. The achenes are provided with a copious pappus and a wide distribution is assured. It is a perennial, and its scarcity seems to indicate that it is of decidedly mesophytic tendencies; it may be considered prophetic in our area. *Pentstemon gracilis*, though rarely a component in any plot, is an interesting form. It is confined to the mesophytic portions of the prairie and has been noted as abundant in western Iowa. Its rarity in our area is explained as in the case of Senecio; it is a perennial, and its smooth though numerous seeds are limited in their distribution. *Lappula texana*, the hairy stick-seed, is also a minor element, occurring mainly on lower slopes in sparse abundance. It is much branched and its numerous small blue flowers exert but a restricted effect. It is an annual and very fertile, producing numerous nutlets whose margins are each bordered by a single row of bristles with recurved tips; distribution is entirely by aid of animals. It continues to bloom well into the estival aspect. The prairie plantain, *P. Purshii*, is a woolly annual whose indeterminate spikes rise some 20^{cm}. Of copious abundance and of gregarious habit, it frequently becomes conspicuous at short distances. Its flowers bloom progressively up the spike, reaching the maximum in the early estival aspect and continuing well into July. Its highly immobile seeds prevent other than a limited distribution, thus readily accounting for its gregarious habit.

The fourth week of the aspect, about the last week in May, is remarkable for the general flowering of *Poa pratensis*. *P. compressa* is associated, but occurs sparsely and principally upon the upper slopes. The relative place of these two forms has been hard to determine, but it seems that *P. compressa* occurs as a forerunner of the bluegrass. In its distribution *P. pratensis* is confined to depressions and lower slopes, and is always indicative of the highest soil-water content, the most favorable exposure, and richest humus. In these situations it forms a dense sod, frequently exclusive, which is constantly pushing up the slopes replacing the bunch-grasses, outlying individuals frequently reaching the higher slopes. It is perhaps one of the most mesophytic of prairie forms, and is almost invariably the forerunner of the prairie shrubs, *Symphoricarpus occidentalis* and *Rhus glabra*. It reaches its highest development on the northwest exposure, and it is up this exposure that the flood-plain and ravine forest of this region has made its greatest advance upon the prairie. Poa is the first facies to bloom, but as noted above is of restricted distribution. It is a perennial and propagation is rapid by the abundant rootstocks, which leads to dense sod.

With the bluegrass are successively added *Oxalis stricta*, *Linum rigidum*, and *Polygala alba*. Never very abundant and of low stature, they add but little to the tone of the aspect, as they are overtopped by the oncoming facies of later aspects. Oxalis appears on lower slopes, possessing frequently a gregarious distribution. It may be either annual or perennial. The few seeds are restrictedly dispersed by the dehiscing capsules. Linum is a decidedly xerophytic annual, being a relict of earlier stages. It appears in the open association on the upper slopes and along prairie crests. It is never common and its fugacious petals prevent all but entire inconspicuousness. Its seeds are few and their mobility little. Polygala is sparingly distributed over the lower slopes of prairie knolls, always working up to higher positions with the increase of mesophytic conditions, but it never remains in the Poa sod. It is perhaps one of the best indices of progressive mesophytism among the prairie species. It is a perennial from woody rootstocks. Seeds are borne two in a capsule and migration is very slow.

As the vernal floral aspect is drawing to a close, several minor elements bloom, extending vernal floral activity over into the first few days of June. *Gaura coccinea, Meriolix serrulata, Psoralea esculenta, Hedeoma hispida,* and *Osmodium molle* now progressively appear. Though flowering during the transition from vernal to estival aspect, these forms reach their maximum display during the early estival; particularly is this true of Meriolix, Hedeoma, and Osmodium. They should be considered transitional species, which appear during the unsettled climatological conditions between the vernal and estival periods, and are not specially indicative of either. Of subcopious occurrence and largely overtopped by leafy stalks of autumnal forms they must be ranked as almost neutral in the floral aspect. *Gaura coccinea* is a relict of more xerophytic stages and occurs primarily though sparsely in the open association of upper slopes and crests. It is an annual and a low and obscure element. It bears a few-seeded indehiscent nut of little mobility. Meriolix may also be considered as a relict of the xerophytic stages of the prairie, having its present distribution limited to the open association along crests and upper slopes, where it frequently occurs subcopiously. Its large yellow flowers make it conspicuous, but only at short range, as low stature relegates it to a sublayer. It is a slightly shrubby perennial from a woody root, producing numerous seeds which are strikingly immobile. It continues blooming up into the autumnal floral aspect, but reaches its maximum in the early estival. Psoralea is rare and of solitary occurrence on middle slopes; add to this its inconspicuous fading-blue flowers and it is scarcely seen upon the prairie by the casual observer. It is a perennial from a large edible farinaceous tuberous root. In the autumnal aspect, breaking off close to the ground, it becomes a tumble-weed. Its rarity is problematical. Hedeoma, a low annual, scarcely ever more than 20^{cm} high, occurs subcopiously and not infrequently copiously upon middle slopes. Its low stature and leafy branches completely hide its abundant small blue flowers, rendering it ever inconspicuous. Osmodium occurs sparsely though quite generally over the whole formation, with the exception of the very crests. It is a perennial with several stalks, terminated by leafy, pendent, scorpioid racemes of greenish flowers, arising from a thick root. It is also quite unnoticeable. The smooth nutlets possess limited mobility.

The estival floral aspect

Marked by a conspicuous decrease of the vernal floral display, a decided climatological change, the flowering of certain sod-formers initiated by *Panicum Scribnerianum* and followed by *Koeleria cristata* and *Stipa spartea*, and the rapid addition of several prominent estival flowers, which all results in a complete shift of the floral tone, the estival floral aspect begins toward the last of the second week in June and extends well up into middle July, when it is terminated by a climatological and floral change even more pronounced.

Toward the end of the vernal aspect the leafy stalks of oncoming facies render the floral tone weak and in places drown it in a sea of dark green; however, the estival forms like Erigeron, Delphinium, Brauneria, Ratibida, Kuhnistera, and others seem to push rapidly above these, forming a higher floral stratum than the vernal, but to be overtopped later on by the still higher stratum of autumnal forms.

With the shifting of climatological conditions, which is very generally appreciated, comes the blooming of *Rosa arkansana*, *Erigeron ramosus*, *Delphinium carolinianum*, and *Brauneria pallida*, which with the sod-formers noted above are always indicative of the inception of the estival aspect. The physical factors of the soil have become less contrasting on the various slopes and between different positions on the same slope; still the south exposure seems slightly earlier. The middle and base of slopes closely approach in absolute water content and are less separated from the crests in this respect than in earlier aspects. Correspondingly there appears a more uniform plant-covering at this time. It is to be noted that the holard is markedly much lower than in the vernal aspect.

During this time Solidago, Helianthus, and Aster have become vegetatively of primary importance and give the general tone of dark green to the aspect. Extending over from the vernal aspect and largely occurring on the upper slopes are *Polygala alba*, which on account of its indeterminate inflorescence blossoms through the entire estival aspect, Meriolix, and *Gaura coccinea*. Hedeoma and *Oxalis stricta* continue blooming on the middle slopes. The dominating species added in this aspect are *Koeleria cristata*, *Ratibida columnaris*, *Symphoricarpus occidentalis*, and *Verbena stricta*. The floral forms added in this aspect are twenty-one in number, nine less than appeared in the vernal aspect. It should also be noted that six new ruderals are added. *Panicum capillare* and *Hordeum jubatum* not infrequently attain no small significance. The latter frequently establishes in waste situations exclusive associations. Its permobile awned spikelets being readily carried by wind or animal, its migration is rapid. In the autumnal aspect *P. capillare* becomes detached at the surface, and the large and profusely branched panicles go tumbling over the prairie until lodgment stops the distribution.

In addition to the floral delineation of the estival from the vernal aspect, it is strongly set off by a change in the climatological conditions. With the introduction of hot dry days and prevailing southern winds the estival aspect begins. The precipitation is slightly higher than in the vernal aspect, but its effect is strongly offset by an average mean temperature some 5° higher than in the vernal. The average hourly wind velocity is 0.9 less and the relative humidity 3.4 lower, the lowest of any aspect. Coupled with these conditions is the fact that the relative evaporation is 1.6 higher, the greatest evaporation of any aspect occurring in the estival.

The holard has a slight rise in the aspect due to the heaviest mean daily rainfall of the season. The chresard thus rises to 11 per cent. on June 6 to fall to 8.9 per cent. on June 22 and 6.5 per cent. on July 7. On June 22 distinction of position seemed nearly eliminated, the base, slope, and crest registering a holard of 15.9, 16.1, and 16.6 per cent.

ESTIVAL CLIMATOLOGY

TEMPERATURE			WIND			RAIN			LIGHT			SOIL			MEAN RELATIVE HUMIDITY	RELATIVE EVAPORATION
Maximum	Minimum	Mean	Prevailing direction	Total movement	Hourly velocity	Days	Mean daily	Total	Mean cloudiness	Sunshine per cent.	Relative intensity	Mean holard	Mean echard	Mean chresard		
35° C.	6 C.	21° C.	S. to E.	5839	8.1	13	.31^{cm}	9.29^{cm}	4.7	56	.551	16%	7.2%	8.8%	48.3	8.794

respectively. Yet the average chresard for the aspect (8.8 per cent.) shows a steady decrease from that of the vernal (10.7 per cent.).

SPECIES OF THE ESTIVAL FLORAL ASPECT

FACIES.—Koeleria cristata,† Poa pratensis.*†

PRINCIPAL SPECIES.—Ratibida columnaris,† Amorpha canescens,† Erigeron ramosus,† Symphoricarpus occidentalis,† Verbena stricta,† Festuca octoflora.†

SECONDARY SPECIES.—Brauneria pallida, Delphinium carolinianum, Rosa arkansana, Linum sulcatum, Panicum Scribnerianum, Meriolix serrulata,* Euphorbia marginata, Potentilla hippiana, Plantago Purshii,* Hedeoma hispida,* Polygala alba,* Hedeoma hispida.*

TERTIARY SPECIES.—Stipa spartea, Aristida purpurea, Allionia linearis, Acerates viridiflora linearis, Osmodium molle,* Anemone cylindrica, Physalis heterophylla, Gaura parviflora, Gaura coccinea.*

RUDERAL SPECIES.—Hordeum jubatum, Ixophorus viridis, Panicum capillare, Melilotus alba,* Lappula Lappula,* Verbena bracteosa, Allionia nyctaginea, Potentilla monspeliensis, Melilotus officinalis,* Lepidium virginicum.*

* From earlier aspect. † Forming associations.

Koeleria cristata is a perennial bunch-grass and a very important sod-former, and may be considered one of the forerunners of the blue-grass. It occurs generally distributed higher up the slopes, where not infrequently it may reach facial rank. Above it seems to be encroaching upon the grama and buffalo grasses and so is quite lacking at the crests. *Panicum Scribnerianum* is likewise a perennial of the bunch habit and is closely associated with Koeleria in distribution, but never appears so abundantly as to become a facies. It is evidently more of a mesophyte than the latter and follows it up the slopes. In the formation studied it is mostly confined to the lower part of the middle slopes. It blossoms slightly before Koeleria. *Stipa spartea* is likewise a bunch-grass and the most xerophytic of these three grasses. It is sparsely distributed upon the uppermost slopes and crests and never forms a facies. It must be rated as an unimportant element in sod-establishment in the formation.

Almost coincident with the sod-formers listed above, blooms the prairie rose, *Rosa arkansana.* It is the first woody perennial to bloom and is distributed over upper slopes and crests where in the latter habitat it commonly becomes copiogregarious. Its abundance, large pink flowers, general anthesis, and height make it always one of the most conspicuous elements of the early estival aspect. Its prominence, however, is of passing duration, as the petals fall after several days and it passes into obscurity. Mobility is limited, resulting largely in the gregarious distribution. With Rosa, blooms *Delphinium carolinianum.* It is a perennial with a thick heavy rootstock, and occurs sparsely as a xerophyte in the open association upon upper slopes and crests. It is closely associated ecologically with the bunch-grasses and invariably seems to follow them. Its single erect stalk, some 6 to 8^{dm} high, bears a large terminal raceme of conspicuous white flowers. Rising thus so conspicuously, these plants seem like sentinels of the prairie and a few individuals are noticeable at some distance. Many seeds are produced, but mobility is slight.

The anthesis of *Erigeron ramosus* usually precedes that of Delphinium but follows that of Rosa, only a day or two separating them. Erigeron is a perennial or annual occurring along upper slopes, where it assumes a copiogregarious habit of growth. It seems quite restricted, few scattering individuals being noted. It is some 7 or 8^{dm} high, with several stalks rising from a single root, which are terminated by spreading corymbs bearing numerous flowers with yellow disks and abundant white rays; thus it is very conspicuous. The achenes are provided with a double pappus, but mobility would seem limited, judging from the gregarious tendency of distribution. Brauneria, though occurring sparsely upon the highest slopes and crests, is one of the most conspicuous early estival bloomers. It is a xerophytic perennial with a large thick root. A single stalk, some 7 or 8^{dm} high, is terminated by a single large head of flowers frequently 5^{cm} across. The numerous long pinkish ligulate ray-flowers surround a large reddish-brown hemispheric head bristling with abundant roughish chaff; in all a very prominent structure. The achenes are crowned with a short-toothed pappus, thus insuring mobility, though of a low degree. It is to be noted that Brauneria has a blooming period of nearly two months, so it remains a conspicuous element even into the following aspect.

Scarcely have these forms flowered, when two species of secondary importance begin to make their contribution to the floral aspect, blooming progressively during the earlier part of the third week of June. They are *Anemone cylindrica* and *Physalis heterophylla.*

These two perennials add little or nothing to the tone of the aspect, as they are of sparse occurrence and decidedly inconspicuous. Anemone occurs rarely though widely on upper and middle slopes. Physalis is of rare occurrence on lower slopes, but would be noticeable except for the pendent inflorescence which hides the yellow flowers beneath the leaves. The former rises some 50 to 60^{cm}, with its exposed cylindrical head of numerous woolly achenes, which are subject to wide dispersal. The immobile fruit of the latter and its ventral position cooperate to insure a very restricted dissemination.

The last days of the third week and early part of the fourth are marked by the general flowering of three forms, *Ratibida columnaris*, *Smyphoricarpus occidentalis*, and *Verbena stricta*, all of primary importance. *Gaura parviflora*, *Linum sulcatum*, and *Allionia linearis*, three flowering forms of minor significance, are added at about the same time. Linum is an annual some 40^{cm} high, occurring sparsely upon the lower slopes. Its humble place in the floral tone is largely due to its limited occurrence, for its yellow flowers (1.5^{cm} in diameter) would otherwise make it a notable element. Gaura is noteworthy mainly as a matter of record, a few specimens only being noted on the middle slope of a northwest exposure. It is an annual and frequently reaches a height of 1^{m} or more. Allionia is a perennial occurring rarely upon middle slopes. The straw-colored involucre incloses one to three small purplish flowers and it is always of minor prominence. The anthocarpous fruit has little mobility. About this time are also added two sod-forming species, *Festuca octoflora* and *Aristida purpurea*. Aristida is of rare occurrence and contributes little to the floral aspect or plant covering. Festuca, on the other hand, is a sod-former of some significance upon lower and middle slopes, being easily replaced however by Poa, which seems to follow it. It is apparently a pioneer form, taking rapid possession of available ground in the open association by means of its heavy, thick, matlike sod.

In Ratibida the long yellow ligulate ray-flowers first spread about June 20, but it is not until several days later that there is a general display and the tube flowers of the columnar disk begin to open. They flower first in a band at the base of the indeterminate head, progressing up at the rate of about 3 to 5^{mm} a day. The plants are some 7.5^{dm} high, and each of the branches is terminated by a very striking head; yellow drooping rays, 6 to 9 in number and frequently 3^{cm} long, surround a deep-brownish columnar head some 4^{cm} high. The prairie cone-flower occurs copiously upon lower and middle slopes, extending crestward (*fig. 1*). In the former locations it frequently assumes almost facial rank and gives a bright-yellow tone to the entire floral aspect. It is a prolific and continuous bloomer, dominating the aspect through the month of July and the greater part of August, and extending up to the middle of September. Ratibida is a perennial from a thick root. The achenes are provided with a diminutive pappus of one or two teeth, and so lack of mobility and great fertility result in its copious abundance in restricted localities. It should be recorded that a few specimens with reddish-brown rays having yellow tips and bases have been noted.

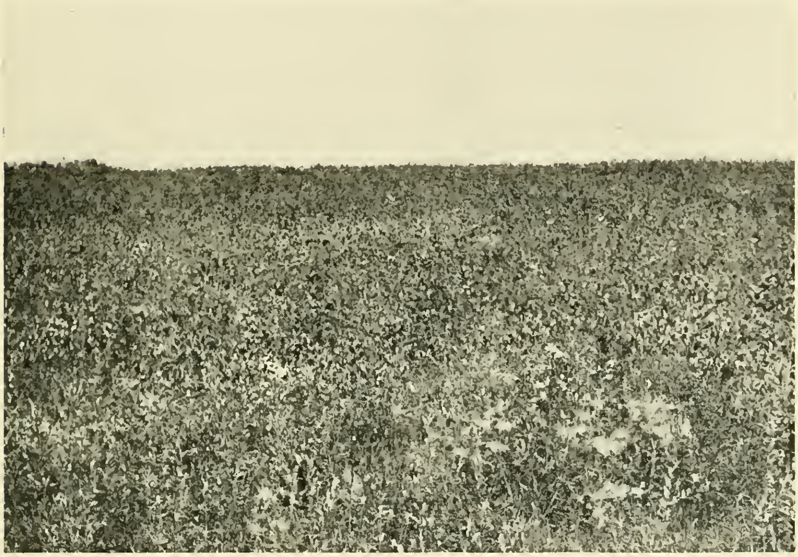

FIG. 1.—Late estival aspect; *Ratibida columnaris* upon a middle slope; the ruderal *Hordeum jubatum* in right foreground.

At about the time Ratibida is spreading its neutral ray-flowers the earlier flowers of Symphoricarpus appear, reaching their maximum flowering two or three weeks later. The wolfberry is of restricted distribution, occurring gregariously at the base of slopes and in mesophytic depressions; frequently outlying individuals are found. In the former situations it ranks not infrequently as a facies. It is the largest of the woody perennials of our area, being a profusely branched shrub frequently 1 to 1.25^{m} tall. It is characteristically associated with the Poa sod, which it follows in the latter's advance upon the prairie; the most advanced occupation is upon the north to northwest exposures, where it also first appears (*fig. 2*). It is the forerunner of *Rhus glabra*, which in other parts of the prairie follows it closely, together making up the shrub stage, which is succeeded by the *Quercus macrocarpa* and *Ulmus fulva* association as the forest pushes out upon the prairie. While the numerous axillary clusters of pink flowers are not conspicuous from a distance, the masses of dark-green leaves make the Symphoricarpus association very noticeable. Very few seeds are borne in the white globular berries;

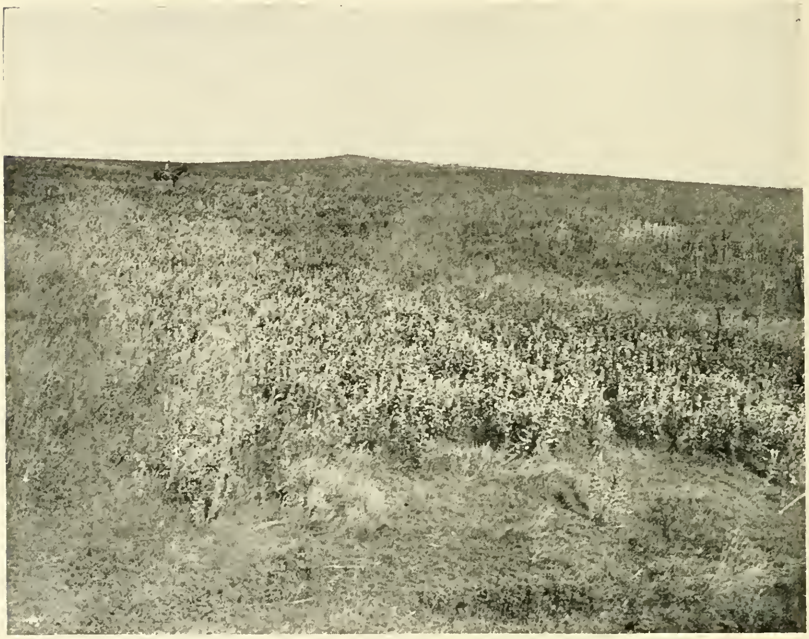

FIG. 2.—Late estival aspect; Symphoricarpus association in a depression of a northwest exposure; Poa sod in foreground.

immobility and high ecological demands result in its gregarious habit. The berries are persistent and birds may help somewhat in dispersal.

The last of this group to bloom is *Verbena stricta.* About June 25 the purplish-blue flowers make their appearance at the base of the long ($15-30^{cm}$) indeterminate terminal spikes in a narrow band, which moves upward day by day at the rate of about 1 to 2^{cm}. The maximum flowering, however, seems to be reached about July 10 to 20.

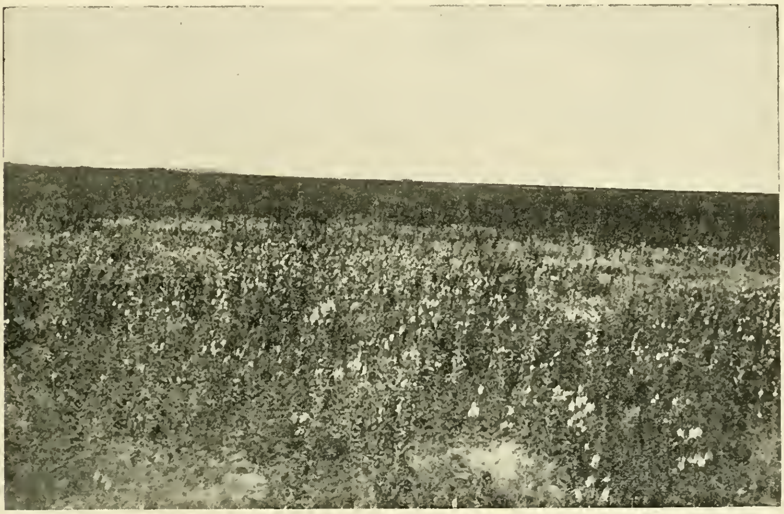

FIG. 3.—Late estival aspect; *Verbena stricta* determining the tone of a lower slope; the white patches are the ruderal *Hordeum jubatum.*

Like Ratibida and for the same reason, it flowers abundantly through July and August and into September. It has a copious and general distribution and not infrequently assumes a dominating influence upon lower (*fig. 3*) and upper slopes as well as crests. It is a perennial from a heavy root and several stalks from the same root give it not infrequently a "bunch" appearance.

During the last days of June and the first days of July, several forms appear to complete the estival floral aspect. In order of flowering they are *Acerates viridiflora linearis*, *Potentilla hippiana*, *Euphorbia marginata*, and *Amorpha canescens.* All assume local importance except Acerates, reaching their maximum display in the serotinal aspect. Acerates appears rarely and as a solitary xerophyte upon the higher slopes and along crests. It is a perennial with numerous permobile comose seeds, yet its abundance is always low. Its solitary umbel of greenish flowers, which blend with the foliage of the prairie, renders its detection difficult. Potentilla possesses a copiogregarious or a solitary distribution along the middle and lower slopes. It rises 50 to 70^{cm}, with the erect stems terminating in loose cymes of numerous yellow flowers. It thus exercises a local effect in the floral aspect. Through its numerous annual rosettes it also contributes in a limited degree to the plant covering. It is a perennial from a thick root. The numerous achenes are highly immobile, resulting in a limited distribution and the gregarious habit. The white-margined spurge, *Euphorbia marginata*, is an annual which occurs subcopiously on lower slopes and rises erect to a height of 50 to 75^{cm}. The stems bear abundant bright-green leaves and are terminated by three-rayed umbels whose greenish-white flowers are subtended by involucres of numerous white-margined bracts, making the entire umbel a very conspicuous object. *Amorpha canescens* is a prominent perennial shrub (50 to 90^{cm} high) exerting a controlling influence. It is a marked xerophyte and may rise to primary rank upon the crests in a sub-copiogregarious distribution, but rarely occurs upon the lower mesophytic slopes. Its gregarious habit and its abundant and densely white canescent leaflets and densely clustered terminal spikes of dark-blue flowers make it a very striking object, especially when it occurs in such abundance. With sod-establishment it gradually disappears, being a characteristic component of the bunch-grass stage. The indehiscent one-seeded pod is highly immobile.

THE UNIVERSITY OF CHICAGO

FLORAL SUCCESSION IN THE PRAIRIE-GRASS FORMATION OF SOUTHEASTERN SOUTH DAKOTA

SEROTINAL AND AUTUMNAL FLORAL ASPECTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 117

LeRoy Harris Harvey

(WITH FOUR FIGURES)

Serotinal floral aspect

Climatologically and florally the serotinal is perhaps the most distinctly demarked of the aspects. The climatological changes which set it off from the estival are quite generally appreciated, while the general blooming of the pioneer sod-formers during the early part of July with the accompanying serotinal bloomers no less distinctly marks it in a floral way.

One records now less dissimilarity in aspect and tone on crest, slope, and base; the more open association, however, still marks the crest. The tone is determined by the dull-greenish vegetative stalks of *Solidago rigida* and *Helianthus Scaberrimus*, which occur copiously throughout the formation. It is relieved locally on lower slopes by the yellow of *Ratibida columnaris* and on upper slopes by the blue of *Verbena stricta* and the canescence of Amorpha; while *Meriolix serrulata*, *Brauneria pallida*, *Potentilla Hippiana*, *Erigeron ramosus*, *Polygala alba*, and *Euphorbia marginata*, all of which extend over from the estival, are less influential. A few conspicuous forms are added in this aspect, but never of sufficient abundance to strongly variegate the dull green of the tone given by the leafy stems of Solidago and Helianthus.

The general flowering of *Agropyron occidentale*, *Bulbilis dactyloides*, and the Boutelouas, together with *Kuhnistera purpurea* and *K. candida*, distinctly marks the inception of the serotinal aspect, which is characterized by the gradual appearance of few but conspicuous bloomers and the attainment of maximum flowering of late estival forms, rather than by the addition of numerous forms as in the

SEROTINAL CLIMATOLOGY

TEMPERATURE			WIND			RAIN			LIGHT			SOIL			MEAN RELATIVE HUMIDITY	RELATIVE EVAPORATION
Maximum	Minimum	Mean	Prevailing direction	Total movement	Hourly velocity	Days	Mean daily	Total	Mean cloudiness	Sunshine per cent.	Relative intensity	Mean holard	Mean echard	Mean chresard		
37 C.	11 C.	24 C.	S.	5682	7.7	10	.25^{cm}	7.95^{cm}	3.8	62	.604	12.4%	7.2%	5.2%	56.8	7.983

vernal and estival aspects, only thirteen new forms appearing during the aspect. It is moreover the aspect of the sod-formers, "floral" forms being represented by only six species. Several ruderals make their floral appearance in the formation during this aspect but, with the exception of *Cassia chamaecrista*, attain no conspicuousness.

The serotinal is markedly a period of extremes, bringing about a change to which one is generally sensible. During the early days of July the weather settles and there is a long succession of long and intensely hot and dry days, which condition characterizes the entire serotinal aspect. Relative evaporation (table) is at an extreme, though slightly less (0.81) than in the estival, finding its explanation in the lower (0.4) hourly wind velocity and higher (8.5) relative humidity; the hot dry winds from the south and southeast are now coexistent with the highest mean temperature, the lowest mean daily precipitation, a low relative humidity, and a high light intensity. Thus evaporation and transpiration are augmented to a precarious degree.

The chresard shows a continued decrease from the estival, being 6.5 per cent. on July 6 and 4 per cent. on July 24; while the average chresard is 5.2 per cent., the lowest yet reached. The holard of crest (8.2 per cent.), slope (12 per cent.), and base (12.2 per cent.) are now (July 28) more nearly approximate than in earlier aspects.

Relative evaporation at a maximum and

the chresard at a minimum are thus the ecological conditions which strongly mark the serotinal floral aspect.

SPECIES OF THE SEROTINAL FLORAL ASPECT

FACIES.—Bouteloua oligostachya,† B. hirsuta,† B. curtipendula,† Bulbilis dactyloides.*†

PRIMARY SPECIES.—Kuhnistera purpurea,† Verbena stricta,*† Ratibida columnaris,*† Kuhnistera candida,† Symphoricarpus occidentalis,*† Amorpha canescens.*†

SECONDARY SPECIES.—Agropyron occidentale, Carduus undulatus,* Euphorbia marginata,* Hymenopappus filifolius, Calamovilfa longifolia, Polygala alba.*

TERTIARY SPECIES.—Lygodesmia juncea, Lacinaria squarrosa, Brauneria pallida,* Meriolix serrulata,* Eriocarpum spinulosum, Erigeron ramosus,* Potentilla Hippiana.*

RUDERAL SPECIES.—Cassia chamaecrista, Onagra biennis, Amaranthus graecizans, Melilotus alba,* Chenopodium album, Lactuca canadensis, Apocynum cannabinum.

*From previous aspect. †Forming associations.

Agropyron occidentale is the first of the serotinal grasses to bloom. It is a xerophytic bunch-grass and occupies prairie crests, where it occurs copiously, rarely even of facial rank. It is one of the pioneers of the bunch-grass stage and is associated with the Andropogons, passing with these forms as they give place to the Boutelouas and being entirely absent in the older and more mesophytic prairie. In transitional stages from the bunch-grass open association to the less xerophytic closed sod association, Agropyron remains not infrequently in subcopious abundance as a relict of the earlier condition. The rootstock is here an efficient mode of propagation.

The three grama grasses, *Bouteloua hirsuta*, *B. oligostachya*, and *B. curtipendula*, which head out during the early days of July, enter upon anthesis almost simultaneously with the beginning of the second week of July, as does also the buffalo grass, *Bulbilis dactyloides*. The Boutelouas are pioneer sod-formers, following only the buffalo grass, which as the pioneer sod-former encroaches upon the bunch-grasses, replacing them and preparing the way for the Boutelouas which invariably follow closely. To the west, where the rainfall is much less, Bulbilis is the prominent sod-former and is the fodder grass of the great cattle ranges west of the Missouri. In our region it occurs along the xerophytic exposures of the bluff line, and as a xerophytic relict along the crest of prairie knolls, where it mingles with the gramas as they advance upon the bunch-grass stage, yet usually as a secondary element among these sod-formers. In our area, however, it is lacking.

Bouteloua hirsuta seems to be the pioneer of the gramas as they encroach upon Bulbilis, or upon the bunch-grasses where Bulbilis is absent, as is the case in our formation. It is in turn apparently followed by *B. oligostachya*, which seems to occur more abundantly and to occupy the most prominent place of the sod-formers. Frequently it alone encroaches upon the bunch-grasses, *B. hirsuta* being absent. Next comes *B. curtipendula* (*fig. 1*), which also contributes largely to the early prairie sod. In many places the Poa sod is the next to follow. The gramas are thus largely confined to the crests, decreasing in abundance downward, where they not infrequently rise to facial prominence. Contrary to the serotinal floral forms, the Boutelouas are of low stature, *B. curtipendula* alone rising above

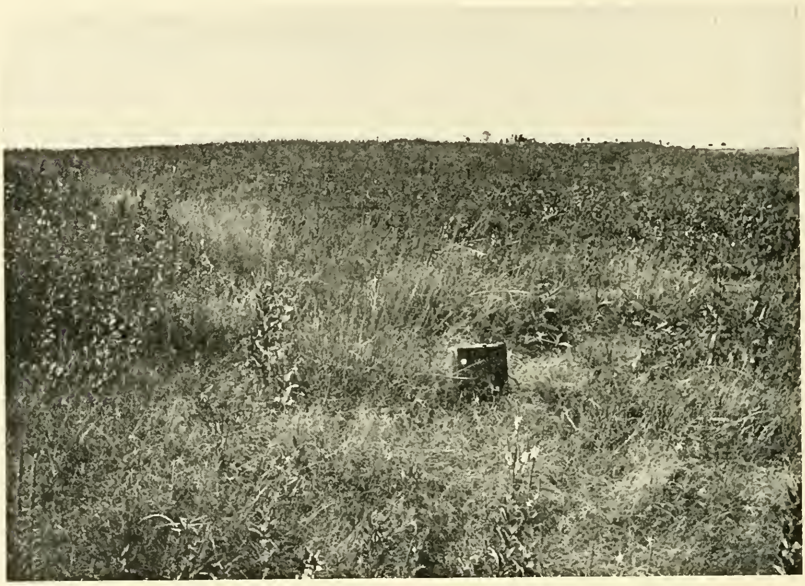

FIG. 1.—Serotinal aspect: *Bouteloua curtipendula* sod on upper slope; *Solidago rigida* to the left; *Helianthus scaberrimus* to the right; admixture in the background.

45^{cm}, which however may frequently reach 60 to 80^{cm}, and is the only species which becomes at all conspicuous. These species as they enter the open bunch-grass association form mats, which fusing form sod, finally resulting in the replacement of the bunch-grasses. The gramas are all perennial by means of the enlarged rootstocks.

Coincident with the flowering of the grasses is that of *Kuhnistera purpurea*, which seems to precede that of its related species *K. candida* by only a few days. The purple Kuhnistera is from its distribution and structure more xerophytic than the white-flowered species. The former occurs most abundantly on the higher slopes, decreasing in abundance downward; while the latter reaches its maximum abundance on the lower slopes, decreasing in the number of individuals per unit area upward. Along middle slopes the abundance of the two species approaches equality. Its distribution seems clearly related to the chresard of these various habitats. CLEMENTS ('05, pp. 233) in light of these facts has suggested the monophyletic origin of these two species from an ancestral form which became split up into *purpurea* and *candida* under the influence of and adaptation to a low and high chresard, a xerophytic and mesophytic habitat respectively, and has instituted experiments to test this theory. In their respective positions of maximum abundance each may rise to dominance, which, however, never occurs in the plot under study; the advanced condition of the prairie seemingly precludes such abundance in the closed association. However, they are the most conspicuous elements of the early part of the aspect. Their branching stalks rise 60 to 80^{cm} and are terminated by cylindrical spikes (some 8 or 9^{cm} long) of white flowers in *K. candida* and (some 5^{cm} long) of violet flowers in *K. purpurea*. They are perennials from thick and deep roots. The seeds are immobile, which with the perennial root accounts for their somewhat even distribution in the formation.

With the prairie clovers appear *Eriocarpum spinulosum* and *Lygodesmia juncea*. Eriocarpum is perennial from a deep woody root, whose much-branched stems rise about 30 to 40^{cm}, terminate in 1 to 25 heads fringed with yellow rays and 2.5^{cm} in diameter. In all it is very striking, but its rare occurrence along upper slopes and crests precludes more than a minor influence upon the tone. It seems to demand only a low chresard, and upon more xerophytic crests than occur in our formation, where it holds its own, it appears copiously and not infrequently determines the tone of the open association. The wind-distribution is facilitated by a copious pappus. Perhaps no plant of our formation has the remarkable degree of adaptability possessed by *Lygodesmia juncea*. The first plant to appear upon the bare exposed soil of bluffs, it persists into a well-formed mesophytic sod with even a marked abundance. It is to be reckoned as a xerophytic relict in our plot, occurring most abundantly along the crest, but at most only sparsely. It is a perennial from a heavy woody root, which interprets its persistence in the formation, and this with its reduced scalelike leaves contributes to its fitness as a pioneer xerophyte. The much-branched stems (45^{cm} high) end in solitary pink-rayed flowers, whose small size and ephemeral duration never render them florally conspicuous. Distribution is very general and wide; the achenes are provided with a copious pappus.

During the last days of the second week and early in the third week of July the prairie thistle, *Carduus undulatus*, enters upon anthesis and florally characterizes this part of the aspect. Its densely white tomentose and much-branched stems rise some 90^{cm} and terminate in large (5^{cm} in diameter) solitary heads of numerous purplish flowers. It also assumes a gregarious habit, and patches occur here and there from base to crest of prairie slopes. Thus it is a most conspicuous form, but reaches its maximum flowering only in early August. Wherever the prairie sod has been disturbed it becomes almost exclusive in its occupancy. It is a biennial of slightly mesophytic tendencies and so appears more commonly on lower slopes. Its high fertility and copious pappus insure a wide distribution, easily explaining its very general occurrence.

The last form to be added in the aspect is *Lacinaria squarrosa*, which appears here and there upon upper slopes and crests. It is very xerophytic in nature, occupying a prominent place in the early stages of the bluff line succession, and is in our plot to be considered as a relict. It is not conspicuous and adds little to the tone, which is at this time rendered bizarre by several of the earlier forms now in their greatest floral display. Lacinaria is an erect (50^{cm}) perennial herb from a tuberous structure, terminating in a spike bearing numerous heads of purplish flowers. The abundant plumose pappus assures a widespread distribution.

Autumnal floral aspect

No marked climatological change is to be noted in passing from the serotinal to the autumnal aspect, but simply one of gradually decreasing favorableness. Hence the latter has been set off partly for convenience of discussion, though it seems more or less distinctly characterized by definite floral activity; yet possibly it might be more accurately designated as late serotinal. Beginning in early August and marked by the estivation of such very conspicuous forms as *Helianthus scaberrimus*, *Solidago rigida*, and the bunch-grasses (*Andropogon furcatus* and *A. scoparius*), it extends into early October, when vegetative activity comes to an end. Its floral activity is terminated, however, in middle September by the flowering of *Gentiana puberula* and *Solidago rigidiuscula*, while florally the aspect is at its best during late August and early September, when the prairie is a sea of yellow from the Solidagos, mainly *S. rigida*, dotted here and there by the blue of Asters. Rising sentinel-like along higher slopes and crests are the rose-purple spikes of the blazing-stars, while on isolated knolls associations of *Aster sericeus* with their purplish flowers and white tomentose leaves relieve the sea of yellow. The bunch-grasses impart a very characteristic tone to the higher and more xerophytic knolls.

In earlier aspects marked restriction of forms was noted, less apparent in the serotinal it is here scarcely evident. On the other hand there is a marked identity of the controlling species on base, slope, and crest, the entire formation presenting an unbroken and identical covering, undoubtedly to be associated with the noticeable equality of the chresard throughout these various situations.

Eighteen of the twenty-two forms (82 per cent.) are composites, and all but one of these (*Kuhnistera villosa*) are wind-distributed. Like the prevernals the autumnals are pronouncedly xerophytic, fitting into uncertain, that is unfavorable, ecological conditions, the former at the initiation, the latter at the decline of floral activity. Just as prevernals have come to possess the spring period of not

AUTUMNAL CLIMATOLOGY

Temperature			Wind			Rain			Light			Soil			Mean relative humidity	Relative evaporation
Maximum	Minimum	Mean	Prevailing direction	Total movement	Hourly velocity	Days	Mean daily	Total	Mean cloudiness	Sunshine per cent.	Relative intensity	Mean holard	Mean echard	Mean chresard		
36 C.	4 C.	20° C.	E. & N.W.	5771	7.8	15	$.26^{cm}$	15.9^{cm}	4.0	63	.607	10.5%	7.2%	3.3%	55.4	4.671

over-favorable conditions, in the same degree the autumnals claim possession of that less suitable period during the closing days of vegetative activity, and for similar reasons. Each group has been able on account of peculiar characteristics to work into and occupy these periods unsuited for plants of less xerophytic tendencies, as their periods of growth and reproduction fitted into these seasons of the year, extremes in each case, where the great majority of forms could not succeed. As floral activity is more definitely restricted than vegetative, which is largely accomplished in earlier periods, it is also evident that the problems of pollination and seed dissemination must have been no small factor in working out through selection the floral restriction of these forms.

The following species extend over from the serotinal aspect, but are rendered inconspicuous by the overtopping layer of a few autumnal species: *Verbena stricta*, *Carduus undulatus*, *Ratibida columnaris*, *Meriolix serrulata*, *Polygala alba*, and *Lygodesmia juncea*. They become less and less conspicuous as the period advances.

As ruderals we may note *Melilotus alba*, coming over from earlier aspects, *Lactuca canadensis*, and *Salsola tragus*.

Conditions here are but the continuation and accentuation, during the early part of the aspect at least, of those of the serotinal. The maximum conditions there noted become less pronounced toward early September, and toward the middle of the month pass gradually into those more settled conditions of early fall in which maturation is so largely accomplished. However, the climatological conditions (table) of this aspect bear to its floral activity a relation analogous to that existing in the prevernal, and the forms here occurring seem in no less degree peculiarly adapted to the late seasonal conditions.

In August the wind is dominantly from the south and east, but in early September begins to swing to the northeast quadrant, where it remains until the prevernal, then swinging round again to the southeast. The average daily rainfall (0.26^{cm}) remains about the same as in the preceding aspect, though it falls on only 25 per cent. of the days, while in the serotinal it falls on 33 per cent. of the days. Relative humidity, sunshine per cent., and relative light intensity are noticeably similar in these two aspects, while relative evaporation has decreased almost a third. In the fact that the hourly wind velocity has increased only a tenth, and in the 4° C. fall in the mean temperature must be sought an explanation of this lowered evaporation.

Species of the autumnal floral aspect

Facies.—Andropogon furcatus,† A. scoparius.†

Principal species.—Solidago rigida,† Aster sericeus,† Helianthus scaberrimus,† Sporobolus brevifolius.†

Secondary species.—Solidago rigidiuscula,† S. missouriensis, S. nemoralis, Ratibida columnaris,*† Aster multiflorus, Artemisia gnaphaloides,* Verbena stricta,*† Carduus undulatus.*†

Tertiary species.—Aster oblongifolius, Solidago canadensis,† Kuhnia glutinosa, Lacinaria scariola, Nabalus asper, Lygodesmia juncea,* Polygala alba,* Dysodia papposa, Grindelia squarrosa, Kuhnia eupatorioides, Lacinaria punctata, Gentiana puberula, Kuhnistera villosa, Meriolix serrulata.*

Ruderal species.—Salsola tragus, Meliotus alba,* Lactuca canadensis.*

*From earlier aspect. †Forming associations.

During the early days of August four forms, destined later to become very conspicuous, make their floral appearance in the following order: *Solidago missouriensis*, *Helianthus scaberrimus*, *Solidago nemoralis*, and *S. rigida*. *S. missouriensis*, the first of the autumnal bloomers, occurs in subcopious abundance and most abundantly in the open association of the upper slopes and crests, where it is quite noticeable, though it never becomes a prominent feature of the tone because of its low stature and small panicles. It is distinctly xerophytic, as is evident both by its structure and distribution, and, as might be inferred, passes as the open formation gradually becomes closed, never being present in a compact sod at the base of the slope.

Appearing with *S. missouriensis* comes *H. scaberrimus.* It occurs both more frequently and abundantly than the former and is distinctly less xerophytic, reaching its greatest abundance upon middle and upper slopes. Its rigid, rough, and little-branched shoots, rising some 1.5^{m} and terminated by a spreading corymb bearing few conspicuous heads (3 to 5^{cm} wide) with numerous yellow rays, make it a conspicuous element of the early autumnal aspect, but it soon blends in the sea of yellow of *Solidago rigida,* which shortly comes on, completely dominating the floral tone. Helianthus (*fig. 2*) rarely holds its own along the tension line where the Poa sod is encroaching, but with other open association forms yields to its advancement, though it occurs inclusively in the less compact sods higher up the slopes, in which places it frequently assumes a copio-gregarious habit. The stiff sunflower is perennial by a thick root-

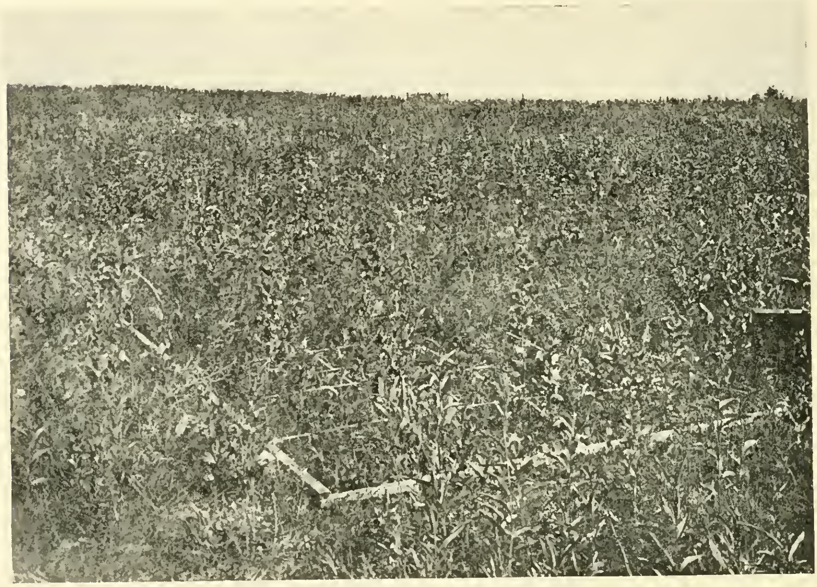

FIG. 2.—Late serotinal aspect: *Solidago rigida* and *Helianthus scaberrimus* on upper slope.

stock. A very limited pappus of two to four scalelike awns provides only a restricted distribution, easily accounting for its gregarious tendency.

About a week or ten days later *S. rigida* begins its blooming, but only reaches its maximum about the last of the month, at this time solely characterizing the floral tone and maintaining a sea of yellow for some three weeks, when the tone begins to pale as fruiting advances and completely gives way during the third week of September, the bright-yellow floral tone yielding to the dull-green foliage. The stiff goldenrod occurs ubiquitously, but reaches its greatest abundance upon middle slopes, where it is frequently copious. The single stout stem (frequently several), from the perennial rootstock, rises somewhat over a meter, terminating in a flat dense cyme bearing numerous yellow-rayed flowers, frequently 20 to 25^{cm} across, which with its abundance and frequency makes *S. rigida* the most striking and dominating in its floral tone of any single species of the formation. Following the latter species by only a few days *S. nemoralis* comes into bloom. Overtopped by and much less abundant than the former, it never is conspicuous, though frequently it adds to the dominant yellow tone of the aspect. It reaches its greatest abundance on slopes, occasionally entering the open association of the crests, but is rarely included in the compact sod of lower slopes.

The three goldenrods just noted are alike perennial from a thickened rootstock, with a tendency toward the formation of perennial basal rosettes, and are widely wind-disseminated through the efficiency of the well-developed parachute.

Almost coincident with the blooming of these four forms is that of the bunch-grasses, *Andropogon furcatus* and *A. scoparius*. Though these two grasses during the last two aspects have been vegetatively conspicuous upon the higher crests and most xerophytic slopes, where they contribute the characteristic dull tone to the bunch-grass association, they flower only during the early part of August, thereby adding but little to their already established prominence. They are accompanied by *Sporobolus brevifolius*, which occurs less abundantly, but like the beard-grasses assumes the bunch habit upon higher crests and ridges. In these situations the Andropogons assume facial rank, *A. furcatus* (*fig. 3*) being the taller and on account of its invariable bunch habit far the more conspicuous. *A. scoparius* (*fig. 4*) seems to form a very loose sod between the bunches of the former and extends lower down upon the slope, where in places it yields to the Boutelouas or rarely to Poa. In succession these forms seem to precede the Boutelouas. With a reduction of drainage and introduction of these sod-formers, the bunch-grasses yield, and in those portions of the prairie where succession has progressed most rapidly, for example the northwestern exposure, the Andropogons and the "bunch-habit" are conditions of the past. With *Agropyron occidentale*, the Andropogons and Sporobolus must be ranked as the pioneer grasses of the prairie, and as such hold a most important ecological relation in the structure and development of the formation. *A. furcatus* yields first, giving way to *A. scoparius*, which in places assumes facial abundance and frequently persists in a somewhat anomalous way in the more mesophytic associations. These grasses

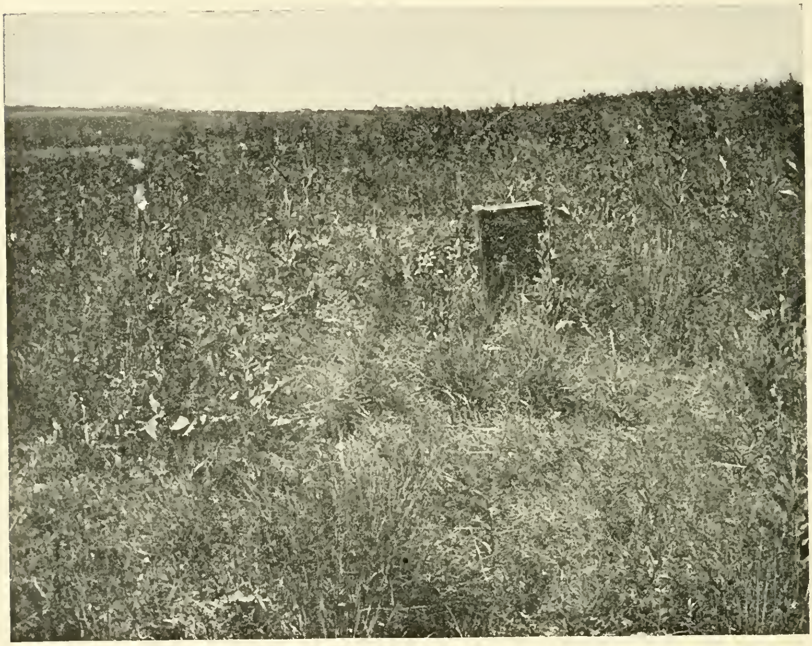

FIG. 3.—Autumnal aspect: the bunch-grass, *Andropogon furcatus*, with intervening spaces occupied by Bouteloua sod near crest of prairie knoll.

are wind-distributed, to which end the hairy awned spikelets contribute. Being perennials from heavy resistant rootstocks, they are well adapted to the precarious pioneer position they occupy in the formation.

Two composites, taking a minor place in the formation, must be mentioned. While belonging to the formation proper, they function mainly as ruderals. *Dysodia papposa*, which blooms at the beginning of the autumnal aspect and often earlier, occurs everywhere along roadsides and on wastes, where it attains its greatest size and abundance. Frequently on the most xerophytic of prairie hills and bluffs it assumes a very marked prominence. In our area it occurs subcopiously along crests and ridges, in fact anywhere that the association may be open, though it is usually depauperate in such cases. The fetid marigold is an annual, and its ubiquity finds cause in the ease and abundance of its dispersal through an efficient pappus, and in the period of its germination falling at the time when the majority of forms have long since germinated and the formation is thinning off its early annuals and prevernal and vernal perennials. To these

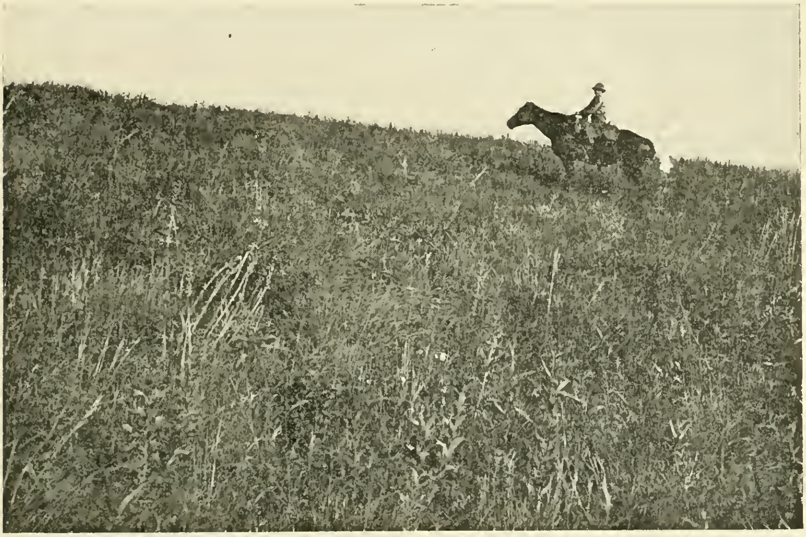

FIG. 4.—Autumnal aspect: an Andropogon sod, mainly *A. scoparius*, upon crest of knoll; scattered individuals of Solidago, Helianthus, and Aster are present.

reasons must further be added its high degree of adaptability for germinating under these less favorable serotinal conditions.

The other form here included is *Grindelia squarrosa*, which occurs only sparsely and then almost entirely along lower slopes and frequently included in the Poa sods. On account of its scattered distribution, the gum plant usually grows about 50^{cm} high and branches much and more or less symmetrically, so that with its many heads of yellow ray-flowers it becomes quite noticeable in early September. Grindelia is a perennial from a heavy rootstock. The few awned achenes are inclosed in a glutinous head, consequently distribution is restricted, and correspondingly a gregarious tendency is to be noted.

During the last two weeks of August several forms of secondary prominence in the floral tone progressively bloom. They are *Solidago canadensis*, *Lacinaria scariosa*, *L. punctata*, *Artemisia gnaphaloides*, *Kuhnia glutinosa*, and *K. eupatorioides*. *Solidago canadensis*, the most mesophytic of our goldenrods, seems to be confined exclusively to Poa sods in valleys and at the base of slopes. It is here gregarious in tendency, occurring in isolated patches or clumps. Also upon disturbed soil around coyote burrows it usually establishes itself in dense patches. It rises about a meter, with several shoots from the same large perennial rootstock, branches profusely, bearing numerous heads in dense panicles, and in all is most conspicuous along with Grindelia at the base of slopes and in valleys. Its distribution by well-developed pappus is extensive, though its demands for the highest ecological conditions greatly restrict its establishment.

The two button-snakeroots, *Lacinaria scariosa* and *L. punctata*, bloom about the beginning of the last week of August, and though sparse in their distribution, being confined to crests, ridges, and the open association of upper slopes, they rise like sentinels of the prairie, relieving the blaze of yellow by touches of purple. *L. scariosa* seems more mesophytic than *L. punctata*, blooming usually a few days later and upon upper slopes mainly. It is also much taller and the heads are larger than in the latter and so more prominent. The numerous short-peduncled heads of purplish flowers, borne upon the erect unbranched and usually solitary stem from a prominent tuber, give these forms a most striking appearance. Copious pappus assures a wide wind-distribution.

The prairie mugwort, *Artemisia gnaphaloides*, is gregarious upon upper slopes. Here it forms dense patches, and these, on account of the white tomentosity of its stems and leaves, which are frequently 50^{cm} high, are conspicuous in the dominant tone of yellow. It bears to the autumnal quite the relation that the Antennarias hold to the prevernal and vernal tone. Perennial from a tuber-like root, it is also xerophytic in tendency. In its pappate achenes and root-propagation are found the causes of its gregarious habit.

The closing days of August are marked by the estivation of the two false bonesets, *Kuhnia eupatorioides* and *K. glutinosa*. They both occur but rarely and then mainly upon upper slopes and crests. They form little clumps (several shoots from the same perennial root) and are tall (50 to 75^{cm}) and much branched, but on account of their small few-flowered heads of creamy-white color and their only occasional frequence, they never attain any prominence. However, they become much more noticeable when the rich white pappus spreads in maturation, during the second week of September. *K. eupatorioides* is more mesophytic and so occurs more frequently over the formation. *K. glutinosa*, however, is pronouncedly xerophytic, and is restricted in its distribution to the open association along crests and higher slopes. The abundant barbulate pappus assures prolific invasion, though establishment seems to be very limited, doubtless due to the apparently low degree of adaptability possessed by these forms.

The Kuhnias are accompanied by *Nabalus asper*, which is restricted to lower slopes, is of rare occurrence and thus always a minor element. Though it is of the upper layer and bears numerous heads of pale-yellow flowers, it blends into inconspicuousness in the general tone. It is a perennial from a tuberous root. A well-developed parachute insures wide dissemination, but high ecological demands preclude more than a rare establishment upon the prairie.

The early days of September are well marked by the blooming of four forms which occasionally attain more or less restricted prominence: *Aster sericeus*, *A. multiflorus*, *A. oblongifolius*, and *Kuhnistera villosa*. They are all of evident xerophytic tendencies and occur mainly upon the upper slopes and crests. Appearing at a time when there is an apparent decline in the dominance of the earlier tone, and occurring in the open association, the Asters become quite noticeable though they are all of a lower layer than *S. rigida*. The silky Aster, *A. sericeus*, usually of low abundance and general occurrence, frequently becomes copious on bunch-grass knolls, there forming very distinct associations. Rising some 50^{cm}, with numerous spreading branches terminating in prominent heads (2 to 3^{cm} in diameter) with numerous violet rays, and bearing abundant leaves which are densely covered above and below with a silvery-white silky pubescence, this Aster is always a conspicuous element of the middle and late autumnal aspect. Its achenes are equipped with a medium pappus. It is a perennial from a thickened rootstock.

The two other Asters are similar in frequency and abundance to *A. sericeus*, but never attain its prominence. Of these *A. oblongifolius* always occurs with *A. sericeus* in the open association. With us it always remains depauperate. Seldom more than 30^{cm} high, it is ever inconspicuous, though its bluish rays make it noticeable at short range, since it is usually overshadowed by *A. sericeus*. It is a perennial and is wind-distributed; a copious pappus serves to bring about a general invasion, which, as in the other Asters, seems to be coupled with a high percentage of establishment, especially in the open xerophytic associations of the formation. The dense-flowered aster, *A. multiflorus*, occurs perhaps more frequently but less abundantly than the former, and is similarly a xerophyte of the open association, in which situations it never reaches other than a reduced stature. However, its bushy spreading branches, thickly beset with numerous small white heads, invariably make it more prominent than *A. oblongifolius*, particularly when it occurs in patches. It seems to work down upon the lower slopes, here attaining a greater stature and abundance as well as a greater prominence in anthesis.

All the Asters are perennial from rootstocks and form small basal rosettes, and are wind-disseminated.

The hairy prairie clover, *Kuhnistera villosa*, is a perennial from a deep tuberous root. Densely silky pubescent, abundantly branched, and terminating in cylindrical clustered spikes (3 to 6^{cm} long) of rose-purplish flowers, it is in itself quite conspicuous, but its restricted frequency and rare abundance make it rarely a tonal component in the formation. In the bunch-grass formation it becomes, with *Amorpha* *canescens*, more abundant but never controlling. It is distinctly xerophytic, and while largely of the open association, it may work down slopes into more favorable habitats.

During the middle days of September the last two forms of the prairie-grass association present their flowers, *Gentiana puberula* blooming a few days before *Solidago rigidiuscula*, the last form to bloom. The downy gentian occurs rarely and is largely restricted to middle and lower slopes, rarely if ever occurring along the xerophytic crests and ridges. Its terminal group of few large light-blue flowers are rendered unimportant as the plant is of short stature (30 to 40^{cm}) and so hidden. It is perennial from thickened roots, and wind-distributed, the seeds being widely winged. Its rarity therefore lies no doubt in its high ecological requirements, being somewhat mesophytic in its nature.

The last form of the prairie to bloom is *Solidago rigidiuscula*. Flowering as it does when *S. rigida* is passing into fruit, of copious abundance and high frequency upon upper slopes, occurring gregariously at times with several stalks (5 to 15) arising from the same perennial root, it is rightly named the "showy" goldenrod. It persists nearly to the middle of October, and is one of the last forms to pass into fruit, though accompanying it are the later flowers of Meriolix, Ratibida, Carduus, Polygala, and Lacinaria. It is widely wind-dispersed and establishment is quite general.

Post-floral aspect

By the second or third week in October the prairie forms of the autumnal floral aspect have all passed into seed, and the gorgeous yellow of *Solidago rigida* has given way to the somber brown of frosted leaves and stalks. While seed maturation and distribution in species of earlier aspects have been in progress during the subsequent aspects, the post-floral aspect, extending up into late November, is particularly characterized by this phase of plant life, yet dispersal may and does continue, but in a much more limited degree even during the winter season. The little fall of snow leaves the prairie bare the greater part of the winter, its tone being in no way modified; the post-autumnal appearance remains to characterize the prairie throughout the non-flowering period, and, as has already been pointed out, extends up to and even dominates the prevernal and vernal floral aspects.

Summary of the structure of the formation

The formation is strictly of the prairie-grass type, its facies being determined mainly by six species: *Bouteloua oligostachya*, *B. curtipendula*, *B. hirsuta*, *Koeleria cristata*, *Andropogon furcatus*, and *A. scoparius*, to which must be added *Poa pratensis* in valleys and on lower slopes. The Andropogons are the main sod-formers of crests and ridges, while the Boutelouas characterize the higher slopes, working up to the crest and ridges. Koeleria seems to be more closely associated with the Boutelouas, occurring on middle slopes mainly. With these are associated three sedges and seven other grasses, which are all important as cooperating sod-formers, some of them ranking as primary species in the formation. We may mention *Carex pennsylvanica*, *C. festucacea*, and *Sporobolus brevifolius* as perhaps the more important of these. It is to be remarked that the sedges are all pre-estival, while the grasses are all estival or post-estival in their floral activity; the first facies to bloom is Koeleria in the estival aspect, while the Boutelouas are serotinal and the Andropogons autumnal.

Upon this facial background of grasses there progressively appear several conspicuous flowered forms of primary importance, which with numerous secondary and tertiary species serve to impart a bizarre aspect to the formation when considered as a whole and a kaleidoscopic shift with seasonal succession. In passing it is well to note that species primary in their own floral aspect may be of only secondary or tertiary importance when the formation is considered as a unit. Among these primary species we may note the ubiquitous *Antennaria campestris*; the Spesias and Sisyrinchium of middle slopes; *Amorpha canescens* upon upper slopes and ridges; Ratibida upon lower, and Verbena and *Erigeron ramosus* upon upper slopes; Symphoricarpus in valleys and on lowest slopes; the Kuhnisteras upon slopes, *K. purpurea* occupying the upper slopes, while *K. candida* extends downward upon lower slopes; *Solidago rigida* and *Helianthus scaberrimus* of great frequency and abundance; and finally *Aster sericeus* upon isolated knolls.

Among the more important of the secondary species may be mentioned *Viola pedatifida* and *Oxalis violacea* of middle and lower slopes; *Meriolix serrulata* of higher slopes; the Lithospermums of the more xerophytic portions of the formation; *Plantago Purshii* of middle slopes; *Linum rigidum* in the open association; *Potentilla Hippiana, Carduus undulatus,* and *Polygala alba,* which occur on middle and lower slopes; *Solidago rigidiuscula* and *S. nemoralis* upon lower and middle slopes, and *S. missouriensis* upon upper slopes mainly; and finally *Aster multiflorus* and *A. oblongifolia.*

Considering the ground association, the open association prevails over ridges and crests and extends down somewhat on slopes, passing gradually through a transitional condition into the closed association which occupies the valleys, depressions, and base of slopes, working always up or outward, displacing the open association. *Poa pratensis* establishes the most dense association, but the Boutelouas, Koeleria, and Festuca exert perhaps a more extensive influence in reducing the open association. In this connection it should be noted that the Andropogons are *par excellence* the pioneers, breaking up the xerophytic open association upon the highest and most xerophytic crests, preparing the way for the Boutelouas. It may be possible that in some cases the Andropogon bunch-grass stage was not the pioneer society, but that on account of more favorable soil moisture conditions, largely a question of drainage, the Boutelouas were the initial sod-formers. However, upon crests and ridges of excessive drainage the Andropogons have invariably preceded the Boutelouas and Koeleria. Occurring rarely in the open association is an undetermined xerophytic moss, while two species of the Basidiomycetes have been noted in the more mesophytic portions of the formation.

The enumeration of species includes 90 forms belonging to the formation proper and some 18 ruderals which work into the formation from the contiguous cultivated regions. The most abundant and prominent of the latter are *Cassia chamaecrista,* two species of Melilotus, *Hordeum jubatum* on lower slopes and moist soil; *Panicum capillare, Verbena bracteosa,* and *Amaranthus graecizans* of the more open associations; *Onagra biennis* of general occurrence; Salsola of the open association; and finally *Lepidium virginicum,* which not infrequently becomes quite abundant in the open association of higher slopes. These ruderals are characteristically confined to marginal invasion, though they are frequently found wherever the open association makes possible their establishment. Onagra, however, is able to establish itself in the closed formation, as is *Potentilla monspeliensis*. Hordeum especially makes advance where some artificial agency has destroyed the equilibrium in rich moist stations; frequently in such cases it assumes even facial rank.

The 90 prairie elements proper have a most interesting taxonomic distribution. The composites with 29 species (32.2 per cent.) form the dominating family, comprising nearly a third of the total forms. The Gramineae number 15 species (16.6 per cent.), and though not leading in species they rank first in number of individuals. The third important family is the Leguminosae with 11 species (12.2 per cent.). Thus these three families provide 61 per cent. of the prairie elements and perhaps over 90 per cent. of the individuals. The remaining 35 species (39 per cent.) are conspicuous on account of their diverse affinities, belonging as they do to 22 different families, 14 of which have only a single representative in the formation. The families are as follows: Borraginaceae (4), Ranunculaceae (3), Cyperaceae (3), Onagraceae (3), Scrophulariaceae (2), Linaceae (2), Rosaceae (2), Oxalidaceae (2); and the following with one species each: Nyctaginaceae, Cruciferae, Umbelliferae, Iridaceae, Violaceae, Euphorbiaceae, Caprifoliaceae, Solanaceae, Labiatae, Plantaginaceae, Gentianaceae, Verbenaceae, and Polygalaceae.

The life conditions of the formation are by no means equable, and in this relation it is significant to record that only 11 per cent. of the species are annual, the majority of which produce abundant seeds and are provided with efficient means of distribution and occur mainly in the xerophytic open associations. Of the remaining 89 per cent. which are perennials, 96.2 per cent. are geophytic; Rosa, Symphoricarpus, and Amorpha alone are woody.

In a region characterized by strong prevailing winds it is to be noted that a high percentage of the species is wind-distributed. An analysis of this point shows that about 90 per cent. are so disseminated, some 55 per cent. showing especial facilities to this end. The great range of specific forms and their marked frequency in the formation finds an explanation in this permobility of reproductive organs possessed by such a large percentage of its components.

As to pollination, 20 per cent. of the forms are wind-pollinated, while 80 per cent. have their pollen transferred by insects, the sedges and grasses comprising the former group.

Conclusions

1. The formation is a part of the Niobrara Prairie Region of CLEMENTS. In composition it is transitional. More truly a part of the prairie to the west, yet it contains several pioneer forms from the more mesophytic prairies to the south and east.

2. These two groups of elements during post-glacial migration have entered along two distinct lines of advance. The former migrated northwestward from a southwestern center of dispersal, while the latter followed a northwestern track up the Mississippi and Missouri valleys.

3. The prairie is pre-glacial in origin and is descended from the climatic prairie of Tertiary times, which arose in response to reduced precipitation caused by the upheaval of the Rocky Mountains at the close of the Cretaceous.

4. The climate is typically a prairie climate. A relatively dry resting season from October to March, in which only 16 per cent. (10.4^{cm}) of the total precipitation falls, and a moist growing season from March to September, in which 83 per cent. (49.31^{cm}) of the precipitation is distributed over sixty days, with 25 per cent. concentrated in April and May, insures a prairie formation. On the other hand, the annual low relative humidity, the dry and high winter winds accompanying high temperature, low winter rainfall, absence of a snow blanket, and the hot, dry summer of low precipitation are inimical to tree growth.

5. The absence of trees upon the prairie is primarily to be explained upon historical lines. The prairie was climatically determined and successfully and successively maintains itself against tree invasion from the edaphically determined arboreal fringes along flood plains and in ravines.

6. The northern slopes are the last to recover from winter, but are most mesophytic. It is up these slopes that the Poa sod and the

shrub association of Symphoricarpus and later *Rhus glabra* advance, preparing the way for the bur oak–slippery elm association, which likewise makes its greatest progress up these slopes from ravines and flood plains.

7. The floral activity of the formation may be approximately recorded in the following five aspects, set off by marked climatic and floral changes: prevernal, April 1 to April 25, 6 species; vernal, May 3 to May 31, 28 species; estival, June 1 to July 7, 21 species; serotinal, July 7 to August 7, 13 species; autumnal, August 7 to September 21, 22 species.

8. The prairie elements show a marked grouping into vertical layers, which correspond approximately with the floral aspects. Overtopped by the autumnal layer the sub-layers are successively those of the serotinal, estival, vernal, and prevernal.

9. There is a marked distinction in the chresard of base, slope, and crest in the prevernal, which becomes less marked in the subsequent aspects, approaching equality in the autumnal. As a result, the floral covering shows a corresponding difference upon base, slope, and crest in earlier aspects; the influence of position gradually declines, the floral covering presenting a striking similarity over the entire formation in the autumnal.

The University of Chicago

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THE MORPHOLOGY OF *PHYLLOCLADUS ALPINUS*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 118

(WITH PLATES XX-XXII)

N. JOHANNA KILDAHL

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(WITH PLATES XX–XXII)

The only account of the morphology of Phyllocladus is that of Miss AGNES ROBERTSON (**18**) in 1906, and this deals exclusively with the anatomical structures and with the affinities of the genus.

The material for the present study was collected by Dr. L. COCKAYNE of New Zealand. It consisted of two collections: one of ovulate and staminate strobili, collected November 1, 1906; the other of ovulate strobili, collected December 11, 1906, from cultivated trees. Unfortunately, the two collections did not afford a very close series, and it was impossible to get at some of the most important points, as the development of the microsporangia, microspores, pollen tubes, female gametophyte, archegonial neck cells, ventral cell or nucleus, and embryo. Dr. COCKAYNE is making collections of the lacking stages at the present time, and it is hoped that a complete description can be presented later. Some material was also obtained from Cambridge, Mass., through the kindness of Dr. B. L. ROBINSON, consisting of three ovulate cones, taken from the Gray Herbarium and collected on the Wilkes expedition in 1838–1842. At what time of the year these cones were collected is not known, but they showed somewhat later stages than those of the last collection made by Dr. COCKAYNE on December 11. It is needless to say that this herbarium material was very much shrunken, and it was impossible to obtain from it any definite structure.

Dr. COCKAYNE's material was killed and fixed in the field, in 70 per cent. alcohol and formalin; cut 5 μ and 7 μ thick; and stained part in safranin and gentian violet, and part in iron alum.

Staminate strobilus

The staminate strobili are formed laterally, in clusters of 2–8 at the tips of the lateral branches. Miss ROBERTSON (**18**) reports one staminate strobilus bearing an ovule at its base, which may indicate

JUN 13 1921

that the ancestral condition was bisporangiate. The sporophyll resembles that of Pinus, although it is comparatively shorter and broader, and has two abaxial sporangia (*fig. 1*). Whether it has any aborted sporangia, as reported in the Taxineae, could not be determined. At least when mature it has fewer sporangia than any of the Taxineae; *Taxus baccata* having seven, *Torreya taxifolia* four, Cephalotaxus four to two, and Phyllocladus two.

Male gametophyte

Material collected November 1 showed the microspores shed and the sporangia wide open along the whole line of dehiscence. Only three or four sporangia retained a few spores, and therefore the sections did not show many stages.

The youngest stage found in the development of the microspore was the uninucleate stage (*fig. 3*), with the wings fully developed. The microspores contain no starch; COULTER and LAND (9) found the microspores of *Torreya taxifolia* rich in starch; Miss YOUNG (20) found starch in the spores of Dacrydium; the body cell of *Cryptomeria japonica* contains starch grains, according to LAWSON (16); COKER (7) found the spores of Podocarpus, during all of the divisions, packed with starch, which disappears just before shedding; CHAMBERLAIN (6) found starch in the spores of *Pinus Laricio*, and Miss FERGUSON (12) in the spores of the species of pine studied by her.

The first division of the microspore cuts off a prothallial cell, which usually lies against the wall, and which immediately begins to disorganize (*fig. 6*). Soon a second prothallial cell is cut off. These two prothallial cells are at first surrounded by delicate walls which very soon disappear, so that when the spore is mature all the four nuclei lie free in the common cytoplasm. The first prothallial cell is commonly evanescent and its nucleus soon disintegrates (*figs. 9, 10*); in two mature spores it was still recognizable (*fig. 11*). The second prothallial cell always persists, in which respect Phyllocladus is similar to Ginkgo.

Prothallial cells have heretofore not been reported in any of the Coniferales except Podocarpineae and Abietineae. COKER (7) reports two prothallial cells in Podocarpus, one of which develops further, while the other slowly degenerates. In *Podocarpus Totarra*

Hallii BURLINGAME (4) finds two prothallial cells, both of which divide and form a tissue, consisting generally of six cells; a similar condition was found in Dacrydium by MISS YOUNG (**16**). Two more divisions follow, so that the mature spore commonly contains four free nuclei—prothallial, tube, stalk, and body nuclei.

On November 1 the four-nucleate microspores are mostly found lodged on top of the nucellus (*fig. 15*); how much earlier they reach it and how long they remain there is not known, as no material previous to this date was available. In exceptional instances pollen tubes had been formed and had penetrated the nucellus (*fig. 18*); but at this date the tube contained only the four nuclei of the mature spore. On December 11 the nucellus was in most cases entirely honeycombed with pollen tubes, and very much disintegrated (*fig. 19*). The pollen tubes do not pass deviously through the nucellus to any great extent, nor branch, but pass almost in a straight line to the embryo sac. As many as fifteen tubes were found in one ovule (*fig. 19*).

Before the pollen tube enters the embryo sac the body cell divides into two equal and optically similar male cells; it was impossible to observe this division, but it takes place some time after the tube has penetrated the nucellus and before it enters the embryo sac (*figs. 20, 31b*). The entire contents of the pollen tube (five naked nuclei) enter the egg (*fig. 20*). ARNOLDI (**1**) also finds that in Cephalotaxus the contents of the pollen tube are emptied into the embryo sac, the contents in this case, however, consisting of only four nuclei; a similar condition has been found in Pinus, where nearly the whole of the contents of the tube is emptied into the cytoplasm of the egg (COULTER and CHAMBERLAIN **10**, and FERGUSON **12**); according to COKER (**8**) this may also take place in Taxodium (**8**) and in Podocarpus (**7**); and COULTER and LAND (**9**) report it for *Torreya taxifolia*. In Cryptomeria LAWSON (**16**) finds that only one male cell enters the archegonium; and JÄGER (**13**) reports the same in *Taxus baccata*. In Phyllocladus it is not uncommon to find the entire contents of pollen tubes within the embryo sac after fertilization has taken place, and even after the eight-nucleate proembryo stage (*fig. 30*); this is no doubt due to the unusual open condition of the archegonia, the greatly disintegrated nucellus (which seems to be of a very mucilaginous consistency), and the large number of pollen tubes.

When the two male cells are equal, it is commonly inferred that both function. So far as could be determined, only one male cell functions in Phyllocladus, and the second male cell, together with the other three nuclei discharged into the egg cell from the pollen tube, disintegrate as the proembryo develops, as shown in *fig. 26*. ARNOLDI (**1**) finds in Cephalotaxus that the second male cell remains in the upper end of the egg and later goes through a mitotic division.

The Taxineae are equally divided in regard to the size of the male cells. In *Torreya taxifolia* they are unequal (COULTER and LAND **9**); they are also reported unequal in Taxus by BELAJEFF (**3**), and by JÄGER (**13**); ARNOLDI reports them of the same size in Cephalotaxus (**1**); and they are equal in Phyllocladus. Outside of the Taxineae they are equal in *Juniperus communis* (NORÉN **17**), Thuja (LAND **15**), Sequoia (ARNOLDI **2**), *Pinus Banksiana* (COULTER **11**), and *Pinus Laricio* (CHAMBERLAIN **6**). Miss FERGUSON (**12**) finds them unequal in *Pinus Strobus;* and one functioning male cell is reported in Podocarpus by COKER (**7**). LAND (**13**) finds in *Ephedra trifurca* that the male cells are equal, both enter the egg, but only one functions.

Ovulate strobilus

The ovulate strobilus occurs on the side of the phylloclad (in the axil of a scale leaf) near its base (*fig. 12*). It usually occurs singlys but frequently in pairs; never more than one pair was seen upon a single phylloclad. The strobilus consists of six to eight rather fleshy scales, each scale bearing a single ovule in its axis.

The ovule has two integuments, entirely free from the nucellu, to the very base. The inner one is very thick and consists of three layers: an inner fleshy, a middle stony, and an outer fleshy consisting of only two layers of cells. The stony layer begins to develop at the micropylar end and extends toward the base. At first it consists of only one layer of cells, but later it becomes much thicker and extends all the way around the base of the ovule, where, however, it remains much thinner than at the micropylar end. The inner fleshy layer is somewhat crowded out by the growth of the stony layer; and the outer fleshy layer is finally sloughed off. The integument contains no vascular strands; these end at the base of the ovule (*fig. 15*); it contains a number of resin ducts, shown in the cross-section in *fig. 16*.

The second integument, called an arillus, arises quite late outside of the heavy integument; on November 1 it appears merely as a small papilla in longitudinal section (*fig. 15*). It grows very rapidly, inclosing the ovule like a cup; the ovule, however, grows up through it, and by December 11 it is merely a light, leathery sheath around the base of the ovule, reaching about half-way to its tip (*fig. 14*).

Female gametophyte

It was impossible from the material in hand to ascertain the development of the female gametophyte, as the formation of walls was in most cases well advanced on November 1. In a few instances it was still in the free nuclear stage (*fig. 18*); and one preparation showed archegonia already formed. The endosperm is of the "ruminating" type, this feature being especially pronounced in the early stages.

The development of archegonia could not be observed, as no stage before fertilization was available. It is impossible, therefore, to state definitely whether neck cells are formed or not. Some indefinite remains of cells, in a few sections, indicate that they exist; and comparing Phyllocladus with the Taxineae, and taking into consideration that neck cells are often destroyed very early by the pushing-in of the pollen tube or the growth of the central cell, the probability is that a two-celled neck exists in the early stages of the archegonium (*fig. 32b*), although nothing was found upon which a definite statement can be based. In *Torreya taxifolia* COULTER and LAND (**9**) found two neck cells; ARNOLDI (**1**) also found two in *Cephalotaxus Fortunei;* JÄGER (**13**) reports two in *Taxus baccata*. From such a condition as that shown in *figs. 19, 31, 32,* it may be inferred that the presence or absence of neck cells would make very little difference. The whole structure of nucellus and gametophyte is of a very mucilaginous composition, and this, together with the prodigality of pollen tubes and male cells, presents a very loose and disintegrated condition. The archegonium in every case, whether located near the surface of the gametophyte or deeper in the tissue, presents an opening as large as the width of the sac. The pollen tube penetrates the tissue, digesting it very readily, and seems to break through the layer of jacket cells surrounding the embryo sac as though no obstacle were in its way. No instance was found, at the stage of fertilization, where the archegonium had not been smashed into, either from the top or from the side, by the pollen tube.

The embryo sac is surrounded by a layer of jacket cells with very large nuclei, and many of the cells are multinucleate. JÄGER (**13**) also pictures a very heavy jacket layer of uninucleate cells in *Taxus baccata;* none are reported in Torreya or Cephalotaxus, although the drawings of the latter by ARNOLDI (**1**) suggest a jacket layer. The archegonia are one to four in number.

No ventral cell or nucleus could be demonstrated, although the probability is that a ventral nucleus is formed; the chromatin in the egg nucleus (*fig. 32b*) before fertilization indicates that it is getting ready to divide. ARNOLDI (**1**) says that in *Cephalotaxus Fortunei* the egg nucleus, shortly before fertilization, cuts off a ventral canal nucleus, which together with a mass of the upper part of the egg destroys the neck cells and passes out of the embryo sac. JÄGER (**13**) does not mention nor picture a ventral nucleus in Taxus; COULTER and LAND (**9**) did not find a ventral cell or nucleus in *Torreya taxifolia;* Miss ROBERTSON (**1**) interpreted a spindle in the central cell of *Torreya californica* as the forming of a ventral nucleus.

Fertilization

At the time of fertilization the egg becomes rich in food vacuoles in the basal end (*figs. 25, 26, 28*). The egg nucleus may be situated near the upper end of the egg (*figs. 22, 23*), or near the basal end (*fig. 28*). The fusion nucleus (*figs. 22, 24*) is partially surrounded by the finely granular cytoplasmic sheath of the male nucleus. This cytoplasmic sheath has been observed in Taxodium by COKER (**8**), in *Torreya taxifolia* by COULTER and LAND (**9**), and in *T. californica* by Miss ROBERTSON (**19**). The non-functional male cell (*figs. 22, 23*), which has begun to disorganize, shows the cytoplasmic sheath very distinctly.

Embryo

The first division of the oospore could not be obtained. Free nuclear division was observed to the eight-nucleate stage. As this was the oldest stage available, it is impossible to say whether more free nuclei are formed or not before the formation of walls. ARNOLDI (**1**) figures ten free nuclei in one section of *Cephalotaxus Fortunei,* and states that there are eight to sixteen free nuclei. Jäger (13) found sixteen free nuclei in the proembryo of *Taxus baccata;* and Coulter and Land (9) found four free nuclei in *Torreya taxifolia.* One of the older ovules showed a long suspensor and an embryo of a few cells buried near the base of the gametophyte (*fig. 33*), but it was impossible to make out its structure.

Summary

The microsporophyll has two abaxial sporangia and the microspores are shed on November 1 or before.

Two prothallial cells are formed, the first of which is generally evanescent; sometimes both persist as free nuclei, the walls of both being evanescent.

The mature microspore has usually four free nuclei, and occasionally five.

The ovulate strobilus is borne on the phylloclad, and bears two to eight ovules.

The ovule has two integuments; one thick and fleshy, the other (arillus) thin and leathery and persistent only at the base. They are free from the nucellus to its base, and contain no vascular strands.

Microspores are found resting on the top of the nucellus on November 1, and occasionally pollen tubes and archegonia are formed at this date.

Pollen tubes are very numerous and pass in a comparatively straight line through the nucellus to the archegonium. They smash the neck cells, if there are any, and empty their entire contents into the egg; the contents of one or more pollen tubes were found in an egg after the eight-nucleate stage of the proembryo.

The body cell divides into two equal male cells just before discharge into the egg; and only one male cell functions.

The archegonium is surrounded by a heavy jacket layer, consisting of multinucleate cells with large nuclei. Two neck cells are probably formed. No ventral cell or nucleus was found, but the material does not justify a definite statement as to its occurrence or not.

The male cell is surrounded by a cytoplasmic sheath which, partly at least, surrounds the fusion nucleus.

At least eight free nuclei are formed by the proembryo before cell walls are formed; and a long suspensor is developed.

This work was carried on under the direction of Professor JOHN M. COULTER; and my acknowledgments are also due to Dr. C. J. CHAMBERLAIN and Dr. W. J. G. LAND, for kindly assistance.

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EXPLANATION OF PLATES XX–XXII

With the exception of *figs. 1* and *2* all the figures were drawn with the aid of a camera lucida and reduced one-half. Index letters are as follows: *e*, endosperm; *p*, perisperm; *n*, nucellus; *if*, inner fleshy layer; *s*, stony layer; *of*, outer fleshy layer; *a*, arillus; *fs*, fleshy scale.

PLATE XX

FIG. 1.—Abaxial view of microsporophyll, showing the empty sporangia.

FIG. 2.—Side view of microsporophyll.

FIG. 3.—Microspore. ×1850.

FIG. 4.—First division of microspore. ×1850.

FIG. 5.—Two-nucleate stage. ×1850.

FIG. 6.—Second division; first prothallial cell against upper wall. ×1850.

FIG. 7.—Three-nucleate stage, showing form of wings. ×3000.

FIG. 8.—Evanescent walls of the two prothallial cells. ×3000.

FIG. 9.—First evanescent prothallial cell lying outside of the cytoplasm of the spore. ×1850.

FIG. 10.—Mature microspore. ×1850.

FIG. 11.—Microspore with both prothallial cells retained. ×3000.

FIG. 12.—Phylloclad with ovulate strobilus.

FIG. 13.—Outer view of fleshy scale with ovule.

FIG. 14.—Inner view of fleshy scale with ovule, showing arillus.

FIG. 15.—Longitudinal section of ovulate strobilus, showing ovules with integuments, fleshy scales, and position of vascular system, November 1. ×40.

FIG. 16.—Cross-section of ovule; archegonia in the center of the endosperm; November 1. ×40.

FIG. 17.—Longitudinal section of the ovule with fleshy scales; micropyle not yet formed; arillus covering the ovule; November 1. ×40.

PLATE XXI

FIG. 18.—Part of nucellus on November 1, showing pollen tubes and part of the female gametophyte in the free nuclear stage; archegonia not yet formed; body cell not yet divided. ×850.

FIG. 19.—Nucellus showing numerous pollen tubes and open archegonia; nearly all the body cells have divided; December 11. ×180.

FIG. 20.—Pollen tube, containing two male nuclei, the stalk and tube nuclei, and one prothallial nucleus, entering the archegonium. ×1850.

FIG. 21.—Archegonial jacket cells. ×3000; *fig. 21a*, same.

FIG. 22. Fertilization: the fusing nuclei are partially surrounded by the cytoplasmic sheath; the second male nucleus lies above the fusing nuclei and has begun to degenerate. ×3000.

FIG. 23.—A later stage in fertilization. ×3000.

FIG. 24.—Fusing nuclei with cytoplasmic sheath. ×1850.

FIG. 25.—Second division of proembryo. ×3000.

FIG. 26.—Four-nucleate stage of proembryo; the other four nuclei degenerating. ×3000.

FIG. 27.—Section through thick integument showing inner fleshy layer, the row of heavy-walled cells (containing crystals) which becomes the stony layer, and the outer fleshy layer of two rows of cells. ×1850.

PLATE XXII

FIG. 28.—Basal end of egg, showing egg nucleus, pollen tube with two male nuclei and one other nucleus, and food vacuoles. ×1850.

FIG. 28*b*.—The archegonium of *fig. 28*, showing position of egg nucleus and pollen tube in the egg. ×100.

FIG. 29.—Basal end of embryo sac, showing four nuclei of the proembryo, remains of the pollen tube, and the non-functional male cell. ×1850.

FIG. 29*b*.—Same as *fig. 29*, showing whole length of embryo sac. ×100.

FIG. 30.—Eight free nuclei of proembryo in basal end of sac, with pollen tube above containing normal contents. ×1850.

FIG. 30*b*.—Same as *fig. 30*, showing top of embryo sac. ×100.

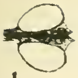
FIG. 31.—Female gametophyte, showing disintegration by pollen tube; the pollen tube is entering the egg from the side. ×100.

FIG. 31*b*.—Same as *fig. 31*. ×1850.

FIG. 32.—This figure shows the straight path usually made by the pollen tubes through the nucellus to the archegonia. ×100.

FIG. 32*b*.—Same as *fig. 32*, showing the two probable neck cells; the loose structure of the chromatin of the egg nucleus intimates that it may form a ventral nucleus; body cell not yet divided. ×1850.

FIG. 33.—Long suspensor, with embryo buried deep in the female gametophyte. ×100.



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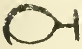

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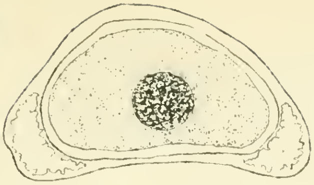

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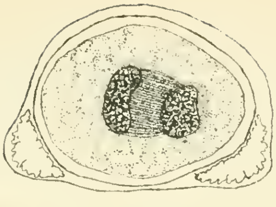

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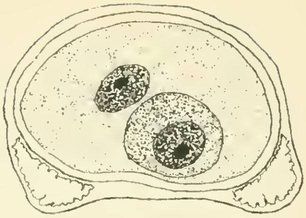

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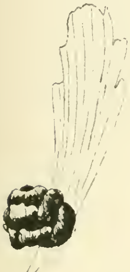

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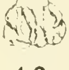

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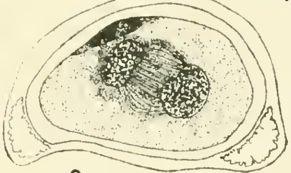

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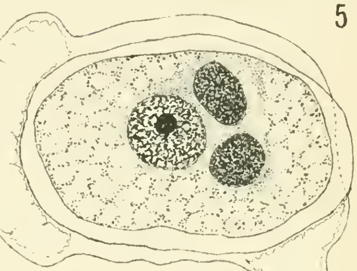

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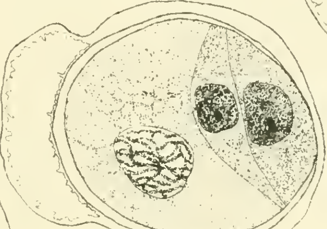

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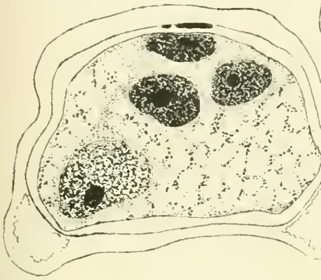

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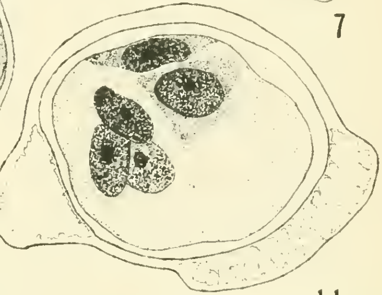

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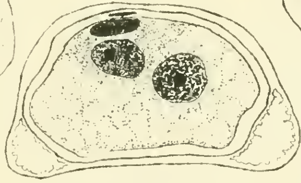

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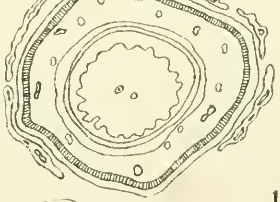

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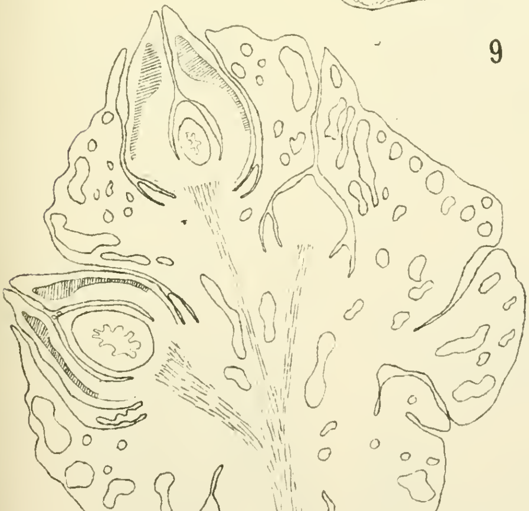

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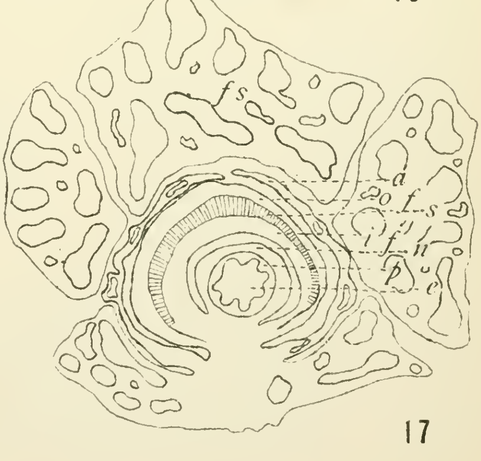

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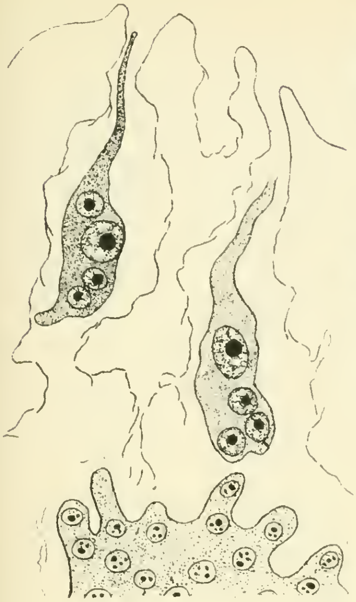
N. Johanna Kildahl Del.

KILDAHL on PHYLLOCLADUS



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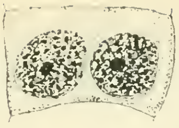

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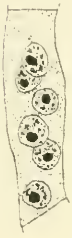

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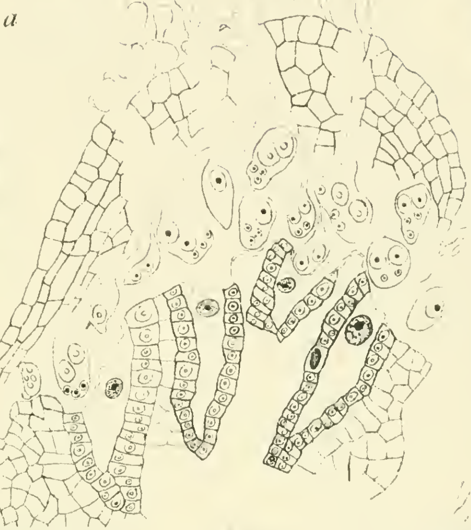

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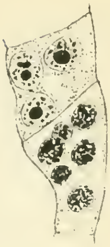

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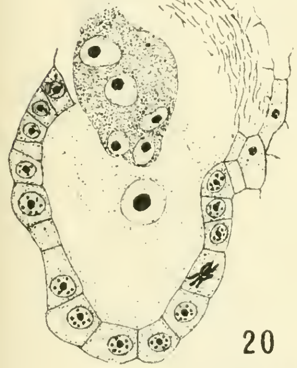

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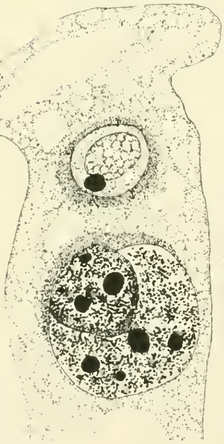

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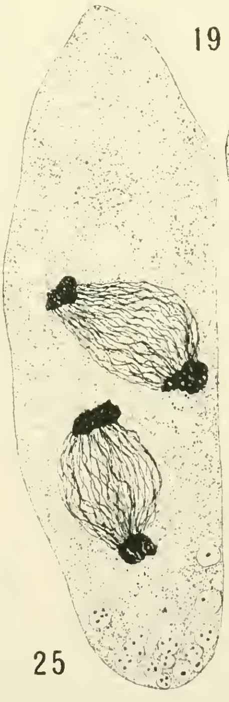

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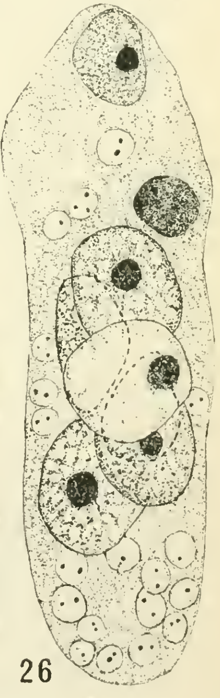

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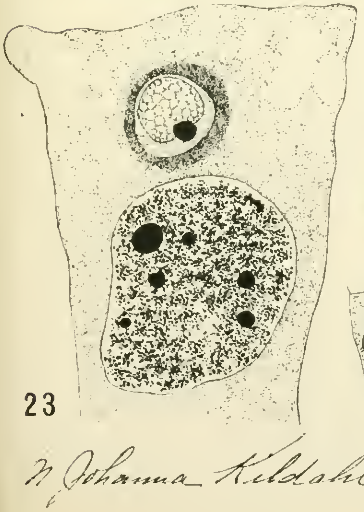

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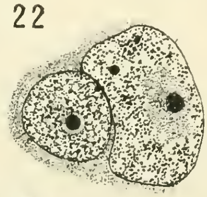

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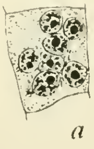

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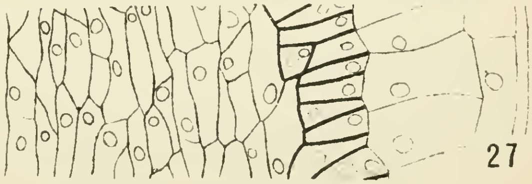

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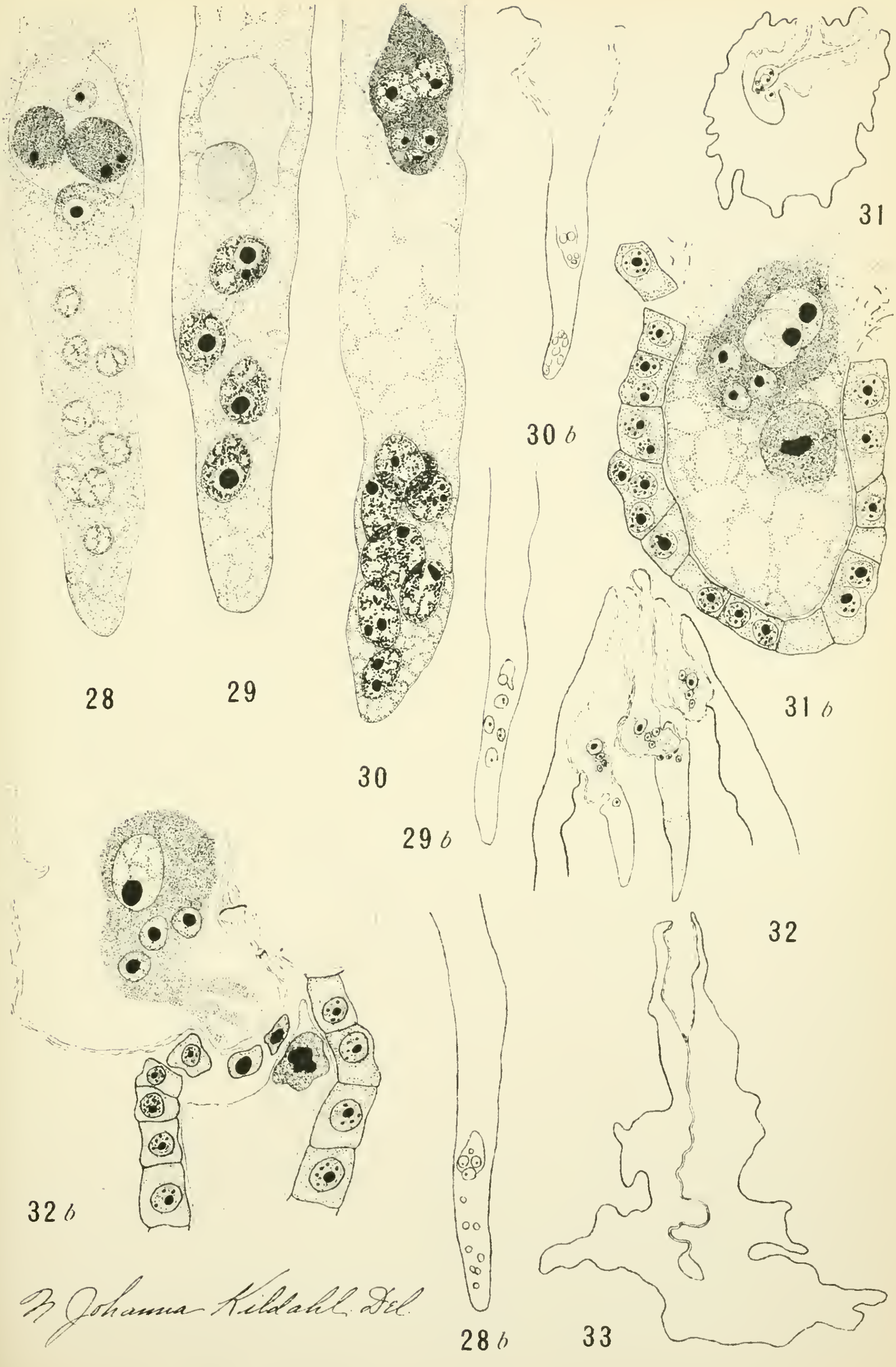
N. Johanna Kildahl. Del.

KILDAHL on PHYLLOCLADUS



N Johanna Kildahl Del.

KILDAHL on PHYLLOCLADUS

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THE VASCULAR ANATOMY OF THE SEEDLING OF *DIOON EDULE*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 119

(WITH PLATES XXIII-XXIX)

REINHARDT THIESSEN

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THE VASCULAR ANATOMY OF THE SEEDLING OF *DIOON EDULE*

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REINHARDT THIESSEN

(WITH PLATES XXIII–XXIX)

This investigation was begun during the winter of 1906, the original purpose being to clear up the confusing statements in reference to the so-called girdling habit of the leaf trace. As the work progressed, its scope became extended, until finally it included all of those anatomical features of the seedling that have a bearing upon the relationship of the Cycadales to the Cycadofilicales and Filicales.

I wish to express my appreciation of the constant encouragement received at all times from Dr. JOHN M. COULTER, under whose direction the work was carried on; I also wish to thank Dr. C. J. CHAMBERLAIN for kindly furnishing the material.

Historical

The first work on *Dioon edule* was by METTENIUS (4), who compared it with *Cycas revoluta*, which was the special form investigated. The methods of those days (1861) did not permit tracing the various vascular bundles throughout their course. Sections were cut only here and there, and the large number of bundles presented only a very incomplete and vague idea to the investigator, resulting in complete misapprehension.

The picture of the leaf trace girdle as METTENIUS drew it is in the main as follows. A bundle in its course toward a leaf divides soon after leaving the central cylinder, the two branches in turn soon dividing. These branches and branchlets, in the main retaining their radially ascending direction, but running at various angles, anastomose with one another and with branches of neighboring bundles, and finally unite with bundles which girdle the vascular cylinder. This girdle lies closest to the ring at a point diametrically opposite the leaf base which its ends enter, each of which therefore traverses the cortex through an arc of about 90°, gradually separating farther from it and

JUN 13 1921

finally entering the leaf base. There is such a girdle for every leaf, and every girdle must cut, on account of its course and location, every other like girdle in two places. Every girdle receives on its inner edge branches from the central vascular bundles which leave the vascular cylinder at various places, and sends out branches from its outer edge to other girdles. From this vague conception, which does not at all agree with the drawings of METTENIUS, the current text-book accounts have been drawn. These accounts, however, do not really interpret METTENIUS, but are as far from his interpretation as that was from the true situation.

In his description of the structure of the bundles METTENIUS was more fortunate, and made a very important contribution. He says that those bundles which are to leave the vascular cylinder are marked off by broad medullary rays and are more definitely bounded than those which continue in the cylinder; that on the inner edge they are provided with spiral vessels (protoxylem), while the others are provided with reticulated cells in the same relative position. At the region of the outward bend of the trace the vascular elements are grouped with the spiral elements on the inner or upper side, immediately bordering the reticulated elements beneath; and this structure the girdle retains in encompassing the stem. Before entering the leaf, however, a change in structure begins to occur, and is completed in the lower part of the petiole; after which the bundle remains unchanged up to the pinnae. The first indication of this change is the appearance of thin-walled cells in the vicinity of the spiral (protoxylem) elements, separating them from the reticulate elements. During the further course of the bundle the spiral (protoxylem) elements gradually move farther within, and the wood is now divided into two parts by the thin-walled cells; the inner part developing its elementary constituents centripetally, and the outer part centrifugally. Finally the spiral (protoxylem) elements are found in the outer part of the bundle, and the centrifugal part is still more reduced, while the centripetal part has reached its maximum development. This structure of the bundle is retained in its further course in the petiole until in the pinnae, where in *Dioon edule* the centrifugal wood is lost altogether. Such is a very brief statement of the description of the transition from centrifugal to centripetal xylem in the leaf traces, as given by METTENIUS. It was the first correct statement of the facts, but their meaning was probably not understood until an interpretation of the situation was given in 1886 by BERTRAND and RENAULT (**5**).

The second paper upon the vascular anatomy of Dioon is that of MATTE (**6**), a brief description of the anatomy of two seedlings being given. In one of them, a very young seedling, the cotyledons were unequal, the larger having four vascular bundles, and the smaller having two small bundles, but with two other very small strands at the very base, one on each side of the other bundles. The bundles continue in a vertically descending course until where the foliar bundles, after being arranged in a circle, have been reduced to four large bundles, separated by medullary rays. The six cotyledonary strands turn now abruptly toward the three poles (protoxylem groups) of the root, converge, and unite two by two in front of them, effecting an entrance through the medullary rays separating the foliar bundles, and unite their phloem with the phloem of the stem. Their secondary xylem unites laterally with that of the stem, while the primary xylem seems to be in direct continuity with the tracheal poles (protoxylem groups) of the root. The centripetal xylem disappears in the passage across the medullary rays.

In the description of the older seedling MATTE found in each cotyledon four bundles of equal size. Their course and method of union is comparable with that found in the other seedling, and they still unite two by two in converging toward the poles (protoxylem groups), but these poles are four in number.

MATTE touches also upon the girdling habit, ascribing it to an intercalary growth produced under the influence of the development of new interior leaves. The vascular strands of the youngest leaves pursue a vertical course, but those of the older ones an oblique course, a comparison of leaves of different ages showing that this departure from a direct course is due to intercalary growth.

Methods

Embryos were removed from mature seeds and killed in chromoacetic acid, imbedded in paraffin according to the usual methods, cut with a rotary Minot microtome, and mounted in series, much care being taken that no sections were lost or misplaced. When only the location or outline of the bundle was wanted, the cross-sections were cut 20 to 30 μ thick; in other cases they were cut 10 μ thick. Longitudinal sections were cut at right angles to the inner surfaces of the cotyledons, as well as parallel to that plane; these were cut 10 μ thick. Staining was done on the slide with safranin and Delafield's hematoxylin, or safranin and anilin blue. Other stains were tried, but these two combinations gave by far the best results. A few specimens were killed by the general picro-mercuric-chlorid method, but no advantage was gained.

Where seedlings were required, the hard coats of the seeds were cut partly open at the micropylar end, and the seeds placed on one side, partially pressed into moist earth, in a flower pot, and put in a warm moist place in the greenhouse and kept well watered. A good many seeds had been planted three years before and had grown to considerable size, some of them having a stem 100^{mm} in length and 25^{mm} in diameter, and bearing several leaves. These were treated about as the embryos, except that all the specimens were cut into lengths of 15 to 20^{mm}. Of course the greater the size of the plant the more slowly the processes of killing, washing, embedding, etc., were conducted. Especial care had to be taken in imbedding the larger specimens, the best results being obtained when they were carried through the process of penetration with paraffin for a whole month. After this time they could be cut with an ordinary Minot rotary microtome with perfect ease, and the sections could be held in complete ribbons. After this precaution, ribbons were obtained from specimens 26^{mm} in diameter and cut 10 μ thick. Staining was done as before with safranin and Delafield's hematoxylin, or safranin and anilin blue, the former combination giving the most satisfactory results. An enormous amount of labor is necessary and also much care to keep sections of entire plants in serial order, both in cross and longitudinal sectioning, but it is the only satisfactory method.

In order to obtain the details or to follow up the leaf trace courses, every section was examined, from the first to the last, and camera drawings made of every second or third section. These were carefully numbered and kept in series and later compared, and reconstructions were based upon them.

Observations

THE EMBRYO

External structure.—In the mature seed the cylindrical embryo is fully two-thirds as long as the endosperm, averaging 20^{mm} in length and about 4^{mm} in diameter. The hypocotyl (*fig. 1*) is comparatively very short, being about 5^{mm} long, ends abruptly (very often is even concave), and in the center is still attached the slender, very much twisted and coiled suspensor (*s*). The cotyledons are free for the upper four-fifths of their length, the lower fifth forming a tubular sheath (*sh*) inclosing the leaf primordia (*figs. 2, 3*). One of the cotyledons is slightly larger than the other, and is inserted a little lower on the axis, slightly enfolding the smaller one. There are generally two leaf primordia, but sometimes three. The broad base of the outer or older one (L_1) embraces the inner and next younger one (L_2), which in turn often embraces a third (L_3); and finally beneath them all is the stem tip (*st*). On the oldest leaf primordia are all the rudiments of the pinnae (*pn*) of the future leaf quite well advanced; while no indication of them can be detected on the younger primordia.

To understand certain features to be described later, it is necessary to note the arrangement of the earlier leaves. An older plant (*fig. 7*) may be used as an illustration, and both scales and foliage leaves will be spoken of as leaves and numbered from *1* to *9* according to age. The cotyledons (*cot*) are apparently exactly opposite; *1* and *2* are approximately opposite, but close observation shows that they are not exactly so; also *1* and *2* are approximately at right angles to the cotyledons (also *fig. 2*). The sheathing leaf base of *1* is a little longer and more slender on the side toward which the spiral turns, and folds around the inner leaf or scale a little farther than it does on the other side. Leaf *3* lacks still more of being opposite *2;* also the corresponding edge of the sheathing base of *2* is more slender and folds over *3* more than its fellow. Leaf *4* lacks still more of being opposite *3*, and again the sheathing base is more slender and folds around the next inner leaves more on that side toward which the spiral turns. In the rest of the series the spiral is uniform, and the overlapping more conspicuous.

Internal structure.—The bulk of the embryo is of course parenchymatous, and the general structure is shown in *figs. 2–6, 8, 9, 29–35.*

The epidermis (*ep*) consists of large, regular, isodiametric cells with large nuclei. That of the cotyledons is abundantly supplied with stomata on the exterior surface of the tubular part and a small portion of the lobes. No stomata occur on the part of the cotyledons remaining in the endosperm, on the interior surface of the cotyledons, or on the primordia. The epidermis of the petioles and bases of the leaf primordia is covered densely with long, unicellular hairs, with well-defined nuclei (*figs. 28, 29*).

The ground tissue is composed of long, regular, prismatic cells, much longer than wide in the cotyledons, but shorter and more isodiametric in the stem proper (*figs. 3, 8*). At the lower extremity of the embryo the cells lose their nuclei, become filled with a dense material, and form a hard capping tissue (*cp, figs. 3, 28, 29*). Distributed irregularly in all parts of the ground tissue are many cells, cavities, and canals filled with mucilage (*m, fig. 8;* black spots in *figs. 28–35*). The cavities are found most abundantly in the cotyledons and are formed by the disorganization of several neighboring cells. The canals, which are formed by the disorganization of the cells end to end, forming tubes of limited length, are most abundant in the petioles of both cotyledons and leaves.

The vascular cylinder is very short, the length being much less than the diameter, and hence it is usually called the vascular plate (*vp*). It is squarish, one diagonal diameter being approximately at right angles to the inner faces of the cotyledons, and the other parallel with them. The xylem (*figs. 32, 33, x*), consisting at this stage probably of protoxylem only, is surrounded by a zone of phloem (*ph*). The xylem is compact and well developed where it borders the phloem, but toward the center it is gradually mixed more and more with pith cells, till in the center the xylem elements lie scattered among the pith cells, these scattered cells being very short and irregular. This condition varies in different specimens, in some the xylem being quite compact to the center, while in others it is entirely wanting in this region (*fig. 32*).

The protoxylem groups.—At each corner of the squarish vascular plate there is a group of protoxylem elements (*px*), which in cross-section is irregularly oval. These four groups extend downward for a short distance, where they form the protoxylem of the primary root (*a, figs. 4, 5, 8*).

The potential vascular tissue.—Above the vascular plate there is a conical or dome-shaped mass of tissue terminating in the growing point of the stem, sharply marked off from cortex and pith, and consisting of long, irregular cells with dense protoplasm and prominent nuclei. The frequent occurrence of mitotic figures shows it to be the most active meristematic region of the stem, being the tissue in which the vascular strands are developed, and therefore the procambium (*pc, figs. 8, 34, 35*).

The pith in the vascular plate contains scattered and short xylem elements (*x, fig. 8*), as stated above. Above the plate it is conical and terminates in a tier of cells against the epidermis (*pt, fig. 8*); in this region the cells are thin-walled and very irregular. In all the pith mucilage cells and cavities are abundant.

The vascular strands.—All the vascular bundles are collateral, except in the upper part of the cotyledons, where they may be regarded as concentric. In the leaf strands the collateral bundle is surrounded by a sheath, which is not well-defined. In the younger strands the few elements are protoxylem, and new elements are added centrifugally (endarch), centripetally (exarch), or in all directions (mesarch). The bulk of the bundle is as yet procambial tissue (compare *figs. 10–15* and *16–21* with *figs. 22–25*). In the older strands it is difficult, if not impossible, to determine where protoxylem ends and metaxylem begins.

The vascular strands of the cotyledons (*figs. 4–6*).—From each of the four protoxylem groups in the vascular plate (*px*) a strand runs outward for a short distance and then branches, the branches separating at wide angles and continuing outwardly in a horizontal plane until well under the bases of the cotyledons, where they turn abruptly upward into the cotyledons, thus giving four strands to each cotyledon, and in such a way that each of the opposite protoxylem groups on the diagonal perpendicular to the inner faces of the cotyledons gives rise to the two inner strands of each of the cotyledons; while each of the other two protoxylem groups gives rise to the outer strands of the cotyledons in opposite edges, that is, one branch goes into the edge of the cotyledon on one side, and the other branch from the same protoxylem group into the corresponding edge of the other cotyledon. This may be stated in another way. In tracing downward, the four strands of each of the petioles of the cotyledons may be said to join two by two. Just before reaching the central cylinder the inner strands of each fuse, and the outer strands of the one fuse with the outer strands of the other, the four strands thus formed giving rise to the four protoxylem groups. This is shown semidiagrammatically in *figs. 4–6*. Tracing these strands farther upward, they are found to branch once more, so that in the upper part of each cotyledon there may be as many as eight strands; but before reaching the tips of the cotyledons they reunite into one concentric bundle (*fig. 15*), which abuts immediately against the epidermis, thus coming into very close contact with the gametophyte. At this place the tissue of the gametophyte is so closely attached to that of the cotyledons that it is difficult to separate them.

The vascular strands of the leaf primordia.—For each leaf or leaf primordium four strands leave the vascular cylinder or vascular plate, at points not definitely located, but quite well distributed, and generally in such a way that approximately one strand for each leaf or primordium leaves on each side of the squarish central vascular cylinder; also those strands belonging to the first leaves have their origin either in the neighborhood of or in the protoxylem groups of the plate. Two strands leave the cylinder approximately on the same side as that on which the leaf for which they are destined is located, and run more or less directly through the cortex into the ventral part of the petiole without further branching; while the other two strands leave the central cylinder approximately on the opposite side and describe a curve around it (the one in one direction and the other in the opposite direction) through the cortex, through the sheathing leaf base, and finally into the dorsal or adaxial part of the petiole, where they branch and rebranch (*figs. 4–6*). It should be emphasized that the point of origin is not at all definite, and that any particular girdle does not describe an arc of any definite extent, but that the length of the arc depends upon the place of origin of the girdle and the position of the leaf to which it belongs.

It has been said that that edge of the leaf base toward which the spiral turns is more slender and folds over the next inner leaf more than does its mate (*fig. 7*). It will be observed that the girdle which is destined for that side of the leaf generally describes a longer curve through the cortex than the one destined for the other side. The phenomenon of girdling will become clearer when illustrated by a specific case. In *fig. 6*, taken from a young seedling, L_1, L_2, L_3, and L_4 represent the first, second, third, and fourth leaves or primordia; L_1^1, L_1^2, L_1^3, L_1^4, the four strands of the first leaf; L_2^1, L_2^2, L_2^3, L_2^4, the strands of the second primordium. L_1^1 is the longest girdle of the first leaf and has its origin at the protoxylem group px^1, on the side directly opposite the leaf to which it belongs, ascends for a short distance, then turns outwardly into the cortex and describes a wide horizontal curve, enters the longer and more slender edge of the leaf base, and ascends in the petiole in the dorsal left-hand portion, branching repeatedly in its further course. Trace L_1^4 leaves the central cylinder near the protoxylem group px^4, ascends a short distance, runs upwardly and outwardly into the cortex, describes a horizontal curve in the opposite direction, enters that side of the leaf having the shorter sheathing base, and ascends in the dorsal or adaxial portion of the petiole, where it branches repeatedly in its further upward course. From this it will be seen that the leaf trace L_1^1 pursues a longer course than trace L_1^4. Trace L_1^2 has its origin near the protoxylem group px^3, to the left, ascends for a short distance, runs out into the cortex, makes a very slight horizontal curve (almost direct) into the leaf base, running outwardly, and then ascends on the ventral or abaxial left-hand portion of the petiole without further branching. Trace L_1^3 has its origin near the protoxylem group px^4, ascends for a short distance, runs outwardly (gradually ascending) with a slight curve into the leaf base, and finally ascends in the ventral or abaxial right-hand portion of the petiole without further branching. It will be seen that in the case of the two inner strands L_1^3 makes a girdle of about 90°, while trace L_1^2 is approximately direct.

About the same condition occurs in the traces of the second leaf. Trace L_2^1 has its origin to the left and near the protoxylem group px^3, ascends vertically for a short distance (but farther than the strands of L_1), runs a short distance outwardly into the cortex and then makes a wide horizontal sweep, enters the leaf base on that side which ensheaths the younger primordium with its more slender edge, ascends in the petioles on the inner or dorsal portion, and branches repeatedly. Trace L_1 has its origin near and to the right of the protoxylem group px^2, describes a girdle of approximately the same magnitude as girdle L_1^4, and runs into the base of the leaf on the right hand. Traces L_2^2 and L_2^3 have their origin respectively on the right and left of the protoxylem group px^1 (quite close to it), and after ascending for a short distance run directly into the outer or ventral portion of the leaf, where they ascend without further branching.

These facts seem to indicate that there is some relationship between the protoxylem groups of the vascular plate and the origin of the trace of the first leaves. The traces of the third leaf (L_3) are followed with some difficulty, but the same condition noted for the first and second leaves is clear. When a fourth leaf is far enough advanced, exactly the same conditions are also presented. The girdling habit of the very young strands is already marked out; but their origin appears no longer to be restricted to the neighborhood of the protoxylem groups, but may occur anywhere in the plate, from which they now ascend vertically for a longer distance before turning out into the cortex. It is very difficult, however, to follow the younger strands while they are still in the potential vascular tissue, since no xylem elements have as yet been formed; but in their passage through the cortex into the primordium their path may be made out clearly, and it shows that the girdle is already established at this stage. Even in the absence of xylem elements, the bundle is clearly marked off by the character and arrangement of its cells (*figs. 26, 27*).

Although the specific case described represents the general state of affairs, many variations are found in the place of origin of the traces. Of the many specimens examined probably no two traces were found to be exactly alike in this respect; also anastomoses between adjoining traces were found here and there. The strands thus ascending vertically for a short distance into the procambial tissue above the vascular plate are the first to assume specific characters. Although the young cylinder is made up of separate traces, it must be observed that these are in the procambial tissue, which is very different from that which surrounds the procambium.

The transition from endarch to exarch xylem.—When a cotyledonary strand leaves the vascular cylinder it is endarch (*fig. 10*). As it passes upward and outward, the protoxylem elements recede from the endarch position and are buried more and more in metaxylem, that is, centripetal wood has appeared. When well up in the tubular portion of the petioles of the cotyledons, the protoxylem is surrounded equally on all sides by metaxylem, the xylem being typically mesarch (*figs. 11–13*). From this point on the protoxylem approaches more and more an exarch position, but the xylem does not become completely exarch. Before the end of the bundle has been reached it has become quite concentric, and it becomes very difficult to determine which element was the first to appear (*fig. 15*). Where the bundle leaves the central cylinder the total xylem elements so far as developed are at the innermost part of the bundle, and as the bundle is traced upward they recede from that position and occupy one farther inward, until in the upper extremity of the cotyledon they occupy a position central to the whole bundle. Thus the transposition of the total xylem holds the same relation to the procambium as the protoxylem holds to the metaxylem (*fig. 9, a*).

The leaf traces also when leaving the vascular cylinder are endarch (*fig. 16*), and in passing outward and upward the protoxylem elements recede from the inner edge and are buried deeper and deeper in the metaxylem; and well up in the leaf bases the xylem has become mesarch (*figs. 18, 19*). Afterward the protoxylem approaches more and more an exarch position, until at the transition between petiole and leaf base the xylem has become completely exarch, with the protoxylem lying immediately against the procambium (*figs. 20, 21*). There is at this stage no centrifugal wood above this point; and there is no secondary wood anywhere. The transition may be said to occur, therefore, between the central cylinder and the leaf base, from which point upward the strands are all exarch. It must be taken into consideration that none of the leaves are as yet fully developed, only the first leaf showing plainly the different regions (*fig. 3*). *Fig. 9, a* represents the situation diagrammatically.

It will be observed that only a very small part of the procambium has been developed into xylem tissue in the whole length of the petioles (*figs. 16, 17*). Cross-sections of foliar strands at a low region show, besides the protoxylem and centrifugal metaxylem elements, a procambium stretching more than half-way across the bundle, the boundary being marked by a thicker-walled tissue, the protophloem. Higher up in the bundle the centrifugal procambium decreases proportionally as the protoxylem moves outward. *Figs. 4, a* and *21* show the situation more clearly; both are taken from very young primordia, the latter much younger and higher up than the former. In *fig. 21* the xylem elements are approximately in the center of the bundle, but there is still considerable procambium between them and the protophloem. The cambium appears to be developed later, just inside the protophloem, and develops tissue actively toward the phloem side only.

THE SEEDLING

EXTERNAL STRUCTURE.—When the seed of Dioon germinates, the hypocotyl pushes through the micropylar end of the seed, where there is at this time an area in the testa, about the diameter of the embryo, which softens readily and is easily penetrated by water. If left to itself, this process takes from a week to a month, and sometimes longer; but if the area referred to is cut away, the hypocotyl pushes through in a few days. During germination the whole of the hypocotyl and the lower part of the cotyledons lengthen.

As soon as the hypocotyl reaches the soil a tap root is sent deep into the ground, and before any leaves appear the root may have penetrated the soil 8 to 12^{cm}, and the diameter of the hypocotyl may have increased to about 1^{cm}. After a long period (which in the greenhouse was about four weeks in case the micropylar end was cut away, but many months if it was not) the first leaf appears between the cleft of the cotyledons, in direct continuation of the axis. As the natural position of the seed during germination is horizontal, and the hypocotyl bends down approximately at right angles, the leaf pushes forth where the cotyledons make a sharp bend. The cotyledons remain in the seed, and in seedlings three or four years old the seed may be seen still attached to the plant. At this time also lateral roots appear in four rows along the primary root, corresponding to the four protoxylem groups.

A second leaf does not appear until perhaps a year later, and about another year elapses before a third appears. A plant of this age has developed about nine or ten leaf primordia, but only about one-third have developed into leaves.

Internal structure.—During germination, in connection with the development of the primary root, not all of the tissue of the tip of the plantlet resumes growth, but only the plerome and a limited portion of the periblem or cortex surrounding it becomes meristematic and pushes through the hard and caplike tissue at the tip of the embryo. The cortex which does not resume growth, as well as the caplike tissue, frays off (*fig. 9, fr*). Also, as growth progresses, the outer layers of the newly formed root, some distance back of the root tip, keeps on fraying off; and underneath a phellogen soon appears and a layer of cork several cells thick is formed. Along each of the four protoxylem strands of the root, continuous from the vascular plate, lateral roots have their origin at definite intervals, being arranged in four rows. Certain cells among and near the protoxylem elements become meristematic, and form the tip of the lateral root, which pushes through the cortex.

The vascular cylinder increases in dimensions uniformly with the growth of the seedling as a whole. New bundles are inserted as new leaf primordia appear, and gradually fill in more and more the vacant spaces between the original strands, so that immediately above the original plate the vascular cylinder is quite compact, while beyond this it continues to be represented by separated strands in the potential vascular tissue.

The vascular strands of the cotyledons.—Not much need be added to the statements in reference to the cotyledonary strands. The transition from endarch to exarch xylem is very much more gradual and the mesarch stage is located relatively much farther up. This is due to the fact that most of the growth of the cotyledon in length occurs at its lower extremity. No secondary wood is developed in cotyledons. The phloem also has increased in bulk, but mature sieve tubes are never developed.

The leaves and scales.—As said above, not all the primordia develop into leaves, most of them remaining abortive and forming scales. Although about one-third of the primordia develop leaves, it does not follow that every third primordium becomes a leaf in regular succession. It sometimes happens that two leaves are developed from consecutive primordia. Evidences indicate that a scale is not predetermined, but remains abortive through some variable cause. The primordia, whether developing scales or leaves, show the same structure in every particular.

The leaf traces.—The course of the leaf traces in the seedling are the same in general plan as those described for the embryo, but the girdling in the older leaves and scales is much more marked. The internal growth and the appearance of new organs has crowded the older parts farther and farther outward. The circumference of the cortex has increased materially, and also the length of the vascular strands running through it. In the older scales and fully developed leaves these are all of about the same extent, and almost horizontal; but from these, through the younger leaves and scales to the youngest primordia, the sweep of the girdle diminishes; but the girdles are already established in the very youngest of the primordia (*figs. 34, 35, ltg.*)

Although in the strands of the youngest leaf primordia no xylem elements are present, the courses of the bundles may be made out readily because of the arrangement, the staining qualities, and shape of the cells, which have denser protoplasm and larger nuclei, and are longer than the adjacent cells (*figs. 26, 27*). The strands of the very youngest primordia which have their origin on the opposite side of the central cylinder show the girdling habit in the same manner as those of the older leaves, but on a smaller scale. The girdle does not always take the horizontal direction, but may be more oblique at the beginning (*figs. 4, 5, 34, 35*). *Fig. 35* shows this clearly, in which *pc* is the procambium and ltg^1 the girdle of the youngest primordium. So long as such a strand is outside of the procambium it can be followed easily, but is lost after it has entered it.

In the oldest plant examined (three or four years old), the first, second, third, and fourth leaves displayed exactly the same condition as was shown in the young leaves of the embryo and seedling. But in the older leaves, outside of these, it is impossible to determine whether the described order is retained, because of the difficulty in following up strands of such size; but it is certain that anastomoses are more frequent, due to the close proximity of crossing bundles.

The transition of the xylem.—The strands of the older leaves have a larger development of metaxylem; also secondary wood may have developed in the lower extremities, so that the transition from endarch to exarch has become more prominent and can be made out with greater clearness. The secondary wood accompanies the bundle as yet only for a short distance, and ceases long before the transition from endarch to exarch is complete. As the secondary xylem and centrifugal metaxylem diminish, the centripetal xylem (which of course is all primary) increases in bulk (*fig. 9, a*). In a plant three years old no other secondary wood was present (*figs. 22, 23*). Even in the oldest leaves of quite old plants the secondary wood, which at the origin of the strand is quite massive, decreases very rapidly, and in the petiole just above the leaf base has thinned out to a few elements (*fig. 24*), remaining quite uniform to the rachis, where it disappears still more; while in the pinna no secondary wood whatever is present, all the xylem being primary and centripetal. Although in the transition region the secondary wood diminishes in the same ratio in which the primary wood increases, it must be noticed that the centrifugal wood is not restricted to the secondary wood alone, as was shown in the younger bundles of the embryo, where the transition is clearly carried out in the protoxylem and metaxylem alone. Thus in the seedlings the transition from centrifugal to centripetal wood is carried on after the appearance of secondary wood, and is completed in the primary wood.

In the older strands where secondary wood has been developed, a considerable amount of the centrifugal wood therefore is metaxylem. This is shown by the amount of procambium that has been developed into centrifugal xylem; as may be seen by comparing the younger strands in *figs. 21, 4, a*, for example, where there is a certain amount of centrifugal procambium, the amount depending upon the distance from the point of egress from the central cylinder, with the older strands in *fig. 24*. Sometimes all of the centrifugal procambium has become xylem; more often, however, patches of procambium or isolated cells of it are never lignified and retain their nuclei, and are then referred to as the thin-walled cells. These thin-walled cells do not necessarily lie against the secondary wood, though they most often do, and become most evident in the upper extremities of the transition. A series of cross-sections of a maturer leaf of a seedling clearly shows the transition from endarch, through mesarch, to exarch, and shows that it is quite independent of secondary wood. The transition begins at the point where the strand leaves the central cylinder, and in the seedling progresses uniformly and is completed in the petiole; but in the old plant the larger proportion of the transition is completed in the lower part of the petiole, where only a few strands of secondary xylem remain and continue uniform until the bundles enter the pinna, when the transition is completed (*fig. 25*).

The cause of girdling.—No particular cause has been assigned for this phenomenon. If such a leaf primordium as is represented in *figs. 3*, L_2, and *8*, L, be selected, it is possible by careful staining to detect four strands which are ultimately developed into vascular bundles. The outer (abaxial) ones pursue a more or less direct course, but in following them from the stem up, their course at first, after leaving the procambium, is vertical; but in the base of the primordium they turn inward to a considerable extent (*fig. 3*, L_2). The two inner or adaxial strands after leaving the procambial cone pursue quite a vertical course, but on reaching a region at the level of the base of the primordium, they begin to turn toward it, one on each side, in some cases ascending rather obliquely, but generally horizontal from the start. *Figs. 34, 35*, ltg^1 shows the girdling of quite a young primordium, the youngest one in that specimen. It is plain, therefore, that the girdle is established very early in the development of the leaf to which it belongs.

Tracheae.—In examining older plants, the oldest one being three or four years old, a singular phenomenon is noticed. Between the various bundles of the stem, which at this time do not yet make a complete ring, vertical connections are found, consisting of irregular, reticulated elements, branching and anastomosing, but forming continuous vessels. In the upper part of the plant, where they are developing, long and narrow cells are found winding and crowding through between the parenchymatous cells. A little farther down in older parts of the stem, these cells are found to be multinucleate, with only here and there an ill-defined cross-wall. A little farther down still the nuclei disappear, and soon lignification appears, developing finally into well-defined reticulated tracheae. These wind in a very tortuous way vertically through the parenchymatous cells from one bundle to another. They are in all senses true reticulated vessels, without cross-walls, establishing a connection between the bundles of the vascular system.

Discussion

In the embryo of *Dioon edule* the vascular cylinder is a protostele, which in some specimens contains a solid xylem mass. From this solid cylinder all gradations are found to the siphonostele. The cells of the pith are actively meristematic, as shown by the mitotic figures, and often in older specimens xylem elements are found in the central part at the level of the vascular plate. The xylem cylinder also continually increases in size, new elements being added to it constantly between bundles already existing, as well as by the cambium.

It should be emphasized that the vascular cylinder in the embryo and seedling does not consist of the short xylem cylinder only, but is continuous in a tissue, very different from the cortex outside and the pith within, which gives rise to strands of procambial tissue running into the leaf primordia. The pith is also a well-defined tissue from the vascular plate to the stem tip, and nowhere suggests that it arises from an intrusion of the cortex through the leaf gaps. An inspection of *fig. 8* shows that it has its beginning at the very tip of the growing point, where it is seen to consist of a single row of cells or a tier of a few cells, gradually expanding as the stem grows into the large pith found in the older part.

The transition of the protoxylem from the endarch to the exarch position was first described by METTENIUS (4), who also suggested the descriptive terms centripetal and centrifugal xylem. The situation was interpreted by BERTRAND and RENAULT (5), who also established that the centripetal wood is in the same relative position throughout its whole length; that it increases in bulk toward its upper end; and that the centrifugal elements are reduced more and more. They say that the centripetal wood is intercalated between the pole and the outer face. It is regarded as the primary wood (developed from the procambium), while the centrifugal wood is regarded as secondary (developed from the cambium).

The present investigation shows that this statement needs modification. While all centripetal wood is primary, all primary wood is not centripetal, as one would interpret from the treatise cited. It has been shown that the transition from endarch to exarch is carried through in the metaxylem, both centripetal and centrifugal wood occurring long before secondary wood is developed. It is very hard to tell where protoxylem ends and metaxylem begins, and where metaxylem ends and secondary wood begins. The separation of metaxylem from secondary wood by means of the thin-walled parenchymatous cells is not a safe guide in the region of transition, and only becomes well marked above this region, where the separation of secondary from primary wood is well marked by the thin-walled parenchymatous cells, as noted by many authors. The metaxylem above this point gets to be relatively very bulky, while the secondary wood is represented merely by a few elements. These were the few pitted cells which presented to VON MOHL (1) a situation without a counterpart, now known to be the herald of the secondary wood which has gradually crept up into the petiole, a transformation begun in its early ancestry, according to SCOTT, the "new wood" driving out "the old," the former being the only wood present in the higher gymnosperms and angiosperms.

MATTE (6) argues in very much the same way as does METTENIUS, an argument which would hold good if the protoxylem and a large part of the metaxylem were left out of account. MATTE says that the bundles of the cotyledons have centripetal wood throughout, centrifugal wood only below the upper region of the petioles, and centripetal and centrifugal wood equally well developed at the bases of the petioles. In the present investigation it has been pointed out that there is no centripetal wood at first, and that it gradually increases; while the centrifugal wood diminishes in bulk to the upper extremities, where it is less than the centripetal but does not disappear entirely.

MATTE further says that what has been said of the cotyledonary traces applies equally well to the foliar traces, except that there is no trace of centrifugal xylem in the youngest leaves. It can be shown that as soon as there are enough xylem elements to show the direction of development the centrifugal wood is present, but gradually disappears, and the centripetal wood increases in the same ratio, until in the upper extremities there is only centripetal wood. This also agrees with BERTRAND and RENAULT, except that their statement that centripetal wood is primary and centrifugal wood secondary does not hold true, a mistake apparently shared by MATTE. It has been shown that protoxylem and metaxylem may have both centripetal and centrifugal elements. This is very well seen in cross-sections at a low level of a young strand, where only a few xylem elements are developed. In such a section the protoxylem lies against the inner edge of the bundle, and the procambium can be seen to occupy considerably more than half of the bundle; the boundary of the procambium and the protophloem is distinctly recognizable, the protophloem forming only about one-third of the bundle. In the upper extremities of the petiole, also, there may be seen at an early stage a considerable amount of procambium outside the protoxylem; as these usually fail to develop xylem, thin-walled cells occur between the primary and secondary wood in the upper extremities (*fig. 21*).

The girdling habit was first noticed and described by KARSTEN (2) in *Zamia muricata*, without, however, giving the definite number of traces. LESTIBOUDOIS (3) adds nothing new except that the traces branch and anastomose. METTENIUS (4) next misinterpreted the situation, as described in the historical introduction (p. 357).

In the embryo and young seedling, at least, the leaf traces pursue definite and well-defined courses and constitute a definite system. Four traces are invariably found to leave the vascular cylinder for each leaf. A few anastomoses occur here and there, but these are always reducible to four strands. On two occasions only five strands were found in one of the cotyledons. In the older seedlings anastomoses are more abundant, but so far as observed these can be reduced to the system found in the young seedling.

When KARSTEN (2) described the girdling habit, he suggested a cause for it in saying that the bundles are formed very early in the young leaf, and that the originally narrow curves are later crowded far out by subsequent growth and the appearance of new organs. METTENIUS (4) also gives a reason for girdling as follows: "In the developmental stage the traces of the youngest leaves lie in the region of the vegetative point, and at first ascend in an almost perpendicular direction, but during the further growth assume gradually an almost horizontal position, and with subsequent growth are lengthened and the expanse is increased." MATTE (**6**) assigns almost exactly the same cause.

A definite cause for the girdling cannot be given at present, for it seems to be deeper seated than at first suspected. The even distribution of the four strands of each leaf in the vascular cylinder appears to be the dominating factor. When the primordium appears on the stem tip, its distance from the potential vascular cylinder is very short, and the same conditions that determine cell division in the development of the leaf, cause the differentiation of cells along certain paths that run from definite points in the procambium to definite places into the developing leaf. After the traces have been started, they continue to develop with the further growth of all the tissues; new organs appear and intercalary growth continues; thus the strands are lengthened more and more and their curves are widened to keep pace with the ever increasing growth of the plant.

Summary

1. The vascular cylinder of the embryo is a protostele, but becomes a siphonostele in the seedling. It is very short and squarish in cross-section, one of the diagonals of the section being at right angles to the inner faces of the cotyledons, and the other parallel with them. Near each of the four corners is a group of protoxylem cells, the long diameter of whose section extends along the diagonal.

2. The four protoxylem groups extend downward to form the protoxylem of the root.

3. From each protoxylem group a foliar strand runs outward for a short distance and then branches, the branches separating at a wide angle and continuing outward until under the cotyledon, when they ascend it; the result is four strands for each cotyledon. The two protoxylem groups on the diagonal perpendicular to the inner faces of the cotyledons give rise to the two inner strands of each cotyledon; while the two protoxylem groups on the diagonal parallel with the inner faces of the cotyledons give rise to the two outer strands of the cotyledons, that is, one branch runs into the outer edge of the one and the other branch (from the same group) runs into the edge of the other cotyledon (opposite the first).

4. For each leaf or scale four strands leave the vascular cylinder

at points not definitely located but well-distributed; two strands of each organ leave the cylinder approximately on the same side as the leaf for which they are destined, and run more or less directly through the cortex into the central or abaxial part of the petiole without branching; while the other two strands of each organ leave the cylinder approximately on the opposite side and describe a wide curve around it, the one in one direction and the other in the other, and finally ascend in the dorsal or adaxial part of the petiole, branching repeatedly. The girdle on the side toward which the spiral may be said to turn is generally the longer one.

5. When the cotyledonary vascular strands leave the vascular cylinder they are endarch, gradually become mesarch in their upward course, and finally approach the exarch condition.

6. The foliar vascular strands also are endarch at their separation from the vascular cylinder, and in their upward course become mesarch and finally exarch. In the very young leaf this transition extends through the whole base and petiole uniformly; but in the adult leaf it is comparatively rapid through the base up to the lower part of the petiole, where only a few centrifugal elements remain, and which remain uniform until in the rachis, where the transition is completed, so that in the pinna only centripetal xylem is left.

7. In the lower stretches of the foliar strands a considerable amount of the centrifugal wood is primary xylem.

8. In the foliar strands of the embryo and seedling, the xylem tissue, so far as developed, from below upward passes from an inner to a central position in reference to the whole bundle.

9. The girdle is established very early, and is horizontal from the beginning.

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EXPLANATION OF PLATES XXIII–XXIX

PLATE XXIII

Fig. 1.—Embryo in mature seed: *cot*, cotyledon; *sh*, tubular sheath of cotyledons; *st*, stem proper; *r*, hypocotyl; *s*, suspensor.

Fig. 2.—Cross-section of embryo just above stem tip: *cot*, tubular part of colytedons; L_1, first leaf; L_1^1, L_1^2, L_1^3, L_1^4, vascular strands of first leaf; L_2, second leaf; L_2^1, L_2^2, L_2^3, L_2^4, vascular strands of second leaf; L_3, third leaf.

Fig. 3.—Median longitudinal section of lower part of embryo, parallel with inner faces of cotyledons: *cot*, cotyledon; L_1, L_2, first and second leaves; *pn*, pinna; *ls*, foliar strand; *st*, stem tip; *p*, pith; *cs*, cotyledonary strand; *vp*, vascular plate; *pl*, plerome; *cp*, caplike tissue; *sp*, suspensor.

Fig. 4.—Semi-diagrammatic reconstruction of part of vascular system of embryo; *cot*, cotyledon; *tb*, tubular part of cotyledons; *cs*, cotyledonary strands; $L_1^1-L_1^4$, foliar strands of first leaf; $L_2^1-L_2^4$, foliar strands of second leaf; *vp*, vascular plate; *a*, protoxylem elements continuing downward into the hypocotyl.

Fig. 4*a*.—Cross-section of a vascular bundle in upper extremity of young leaf; *x*, xylem elements; *a*, cells losing their contents during lignification; *pc*, procambium; *pph*, protophloem; *b*, line showing boundary between protoxylem and protophloem.

PLATE XXIV

Fig. 5.—Semi-diagrammatic reconstruction of part of vascular system of embryo, to show especially the girdling: L_1, L_2, traces of first and second leaves; *vp*, vascular plate; *px*, protoxylem groups; *a*, xylem elements continuing down from protoxylem groups to form the protoxylem of the primary root.

Fig. 6.—Diagram giving bird's-eye view of vascular system, to show origin and girdling of foliar strands: *cot*, tubular part of cotyledons; *cs*, cotyledonary traces, one group for each cotyledon; L_1, L_2, L_3, L_4, first, second, third, and fourth leaves; L_1^1, L_1^2, L_1^3, L_1^4, traces of first leaf; L_2^1, L_2^2, L_2^3, L_2^4, traces of second leaf; *px*, protoxylem groups.

Fig. 7.—Outline of cross-section just above stem tip of seedling three or four years old, to show phyllotaxy; *cot*, cotyledons; *1–8*, leaves in order of age.

Fig. 7*a* (1).—Stem tip from germinating embryo with leaf primordium (*L*) developing on the side.

FIG. 7*a* (2).—Stem tip from rapidly growing seedling, showing growing point (*st*) in the middle and a leaf primordium (*L*) on each side; this section showed numerous spindles in growing point and primordia.

PLATE XXV

FIG. 8.—Details of central region of embryo in median longitudinal section: *L*, youngest leaf; *st*, stem tip; *ep*, epidermis; *pt*, pith terminating in stem tip; *pc*, procambium; *vp*, vascular plate; *ph*, phloem; *a*, continuation of protoxylem into the root; *p*, pith; *pl*, plerome; *m*, mucilage cells.

FIG. 9.—Median longitudinal section of lower part of germinating embryo, showing lengthening of plerome, development of primary root, and fraying-off of epidermis and outer part of cortex (*f*).

FIG. 9*a*.—Diagram to show transition of protoxylem in the metaxylem, and transition of primary xylem from an inner to a central position in a young bundle: *aa'*, inner, and *gg'*, outer limits of bundle; shaded portion (*ab*, *cd*), xylem tissue, *ad*, protoxylem; unshaded portion in *aa'* (*cj*), procambium; *cj* (*gg'*), protophloem; dotted line *cj*, boundary between procambium and protophloem.

PLATE XXVI

FIGS. 10–15.—Series of cross-sections of cotyledonary traces: *fig. 10*, just before joining vascular cylinder; *fig. 15*, after the several bundles have reunited in upper extremity of cotyledon; *px*, protoxylem. ×850.

PLATE XXVII

FIGS. 16–21.—Series of cross-sections of leaf traces: *fig. 16*, just before joining vascular cylinder; *figs. 17–20*, at considerable intervals above each other; *fig. 21*, younger trace; shaded portion protophloem. ×850.

PLATE XXVIII

FIG. 22.—Cross-section at low level of foliar trace of a three-year-old plant: *px*, protoxylem (endarch); *x*, xylem, of which probably the larger portion is centrifugal metaxylem and only a small portion is secondary xylem; *ph*, phloem; *pph*, protophloem. ×425.

FIG. 23.—Section from the same strand as *fig. 22*, but considerably higher up; *px*, protoxylem (mesarch). ×425.

FIG. 24.—Section of bundle from old leaf of quite a large plant just below rachis: *px*, protoxylem (exarch); *mx*, metaxylem (centripetal); *tc*, thin-walled cells; *sx*, secondary xylem; *ph*, phloem; *pph*, protophloem. ×425.

FIG. 25.—Cross-section of bundle in pinna: lettering as in *fig. 24;* note absence of secondary xylem. ×425.

FIG. 26.—Median longitudinal section of very young leaf trace, showing shape and arrangement of cells; shaded portion, earliest procambium. ×1200.

FIG. 27.—Cross-section of the same bundle as in *fig. 26*.

PLATE XXIX

FIG. 28.—Median longitudinal section of embryo at right angles to inner faces of cotyledons. ×11.

FIG. 29.—Median longitudinal section similar to *fig. 28*: *ct*, cotyledonary traces; *cp*, caplike tissue; *s*, suspensor. ×30.

FIG. 30.—Cross-section of young seedling at region of union of cotyledonary trace with vascular cylinder: *ct*, cotyledonary traces belonging to one cotyledon. ×16.

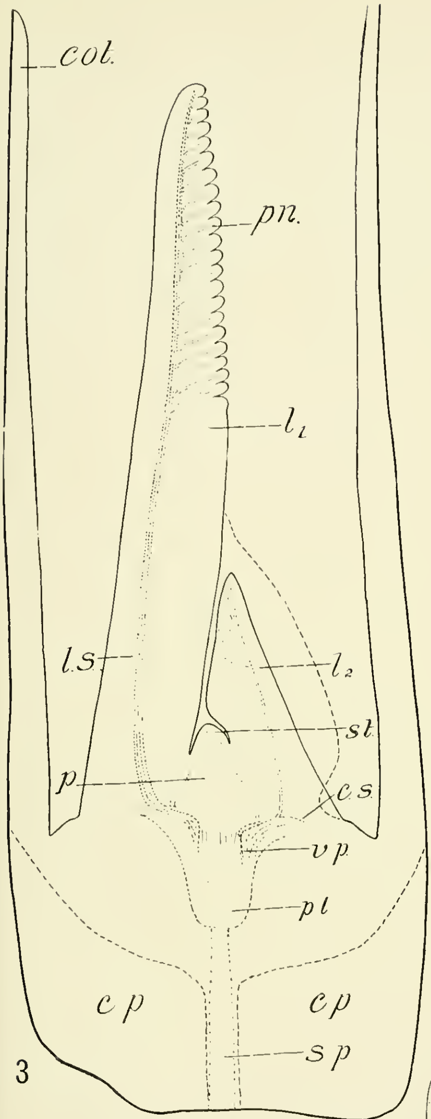
FIG. 31.—Section of same further up to show girdling: L_2^1, outer long girdle; L_2^4, outer shorter girdle; L_2^2, L_2^3, inner girdles; all of second leaf. ×16.

FIGS. 32, 33.—Cross-sections of embryo at region of vascular plate (*vp*); *px*, protoxylem; *x*, xylem; *ph*, phloem; union of cotyledonary traces two by two, and union of resultants with vascular plate at protoxylem groups. ×30.

FIG. 34.—Median longitudinal section of seedling three years old: *pc*, procambium; *ltg*, girdle; *lt*, trace; *lg*, leaf gap. ×30.

FIG. 35.—Cross-section of embryo to show early appearance of girdle; *pc*, procambium; ltg^1, girdle of very young leaf; ltg^2, girdle of next older leaf. ×30.

The black spots in all the photographs are mucilage cavities or cells.



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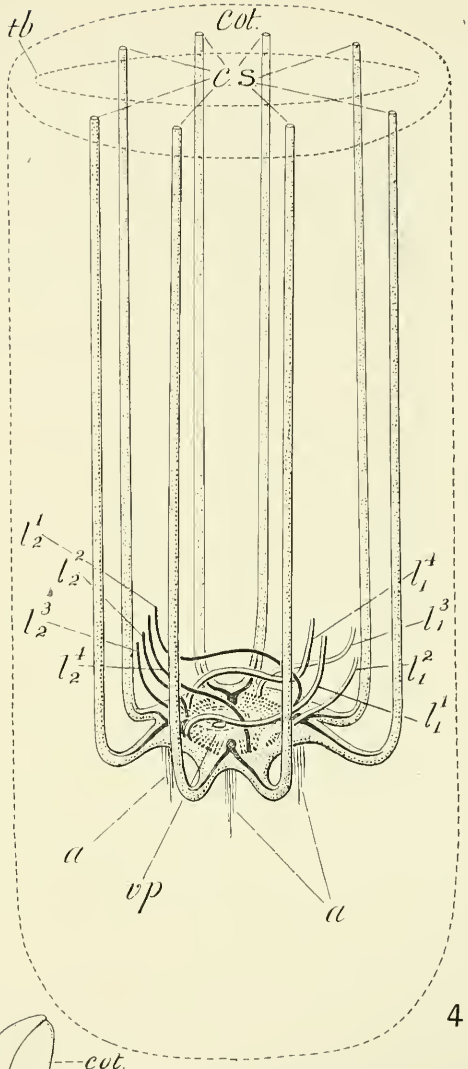

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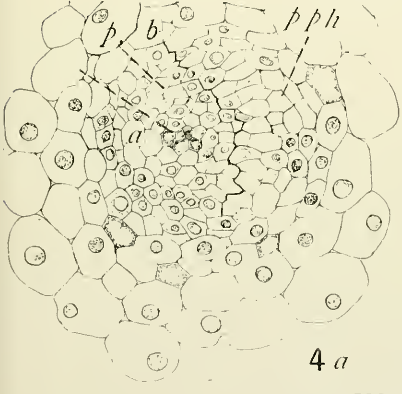

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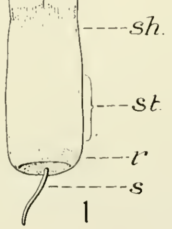

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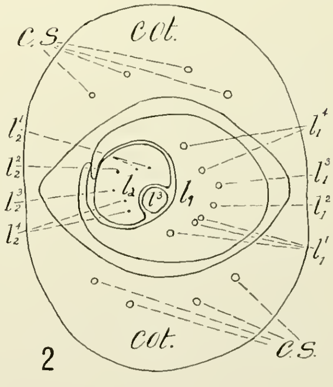

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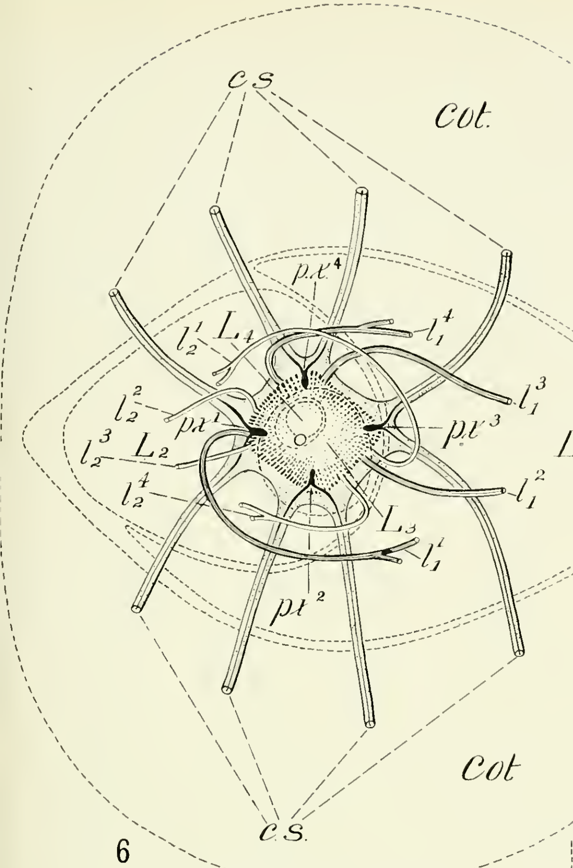

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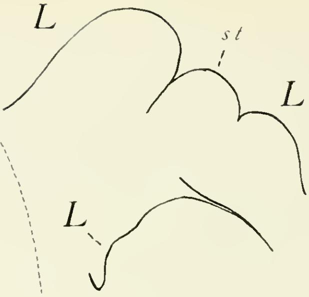

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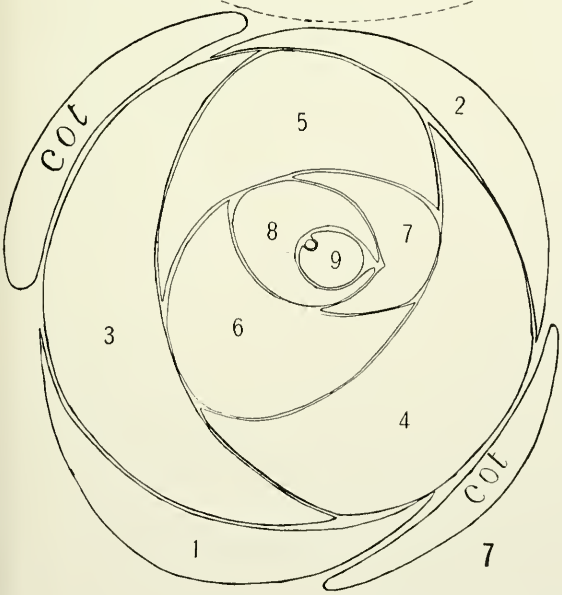

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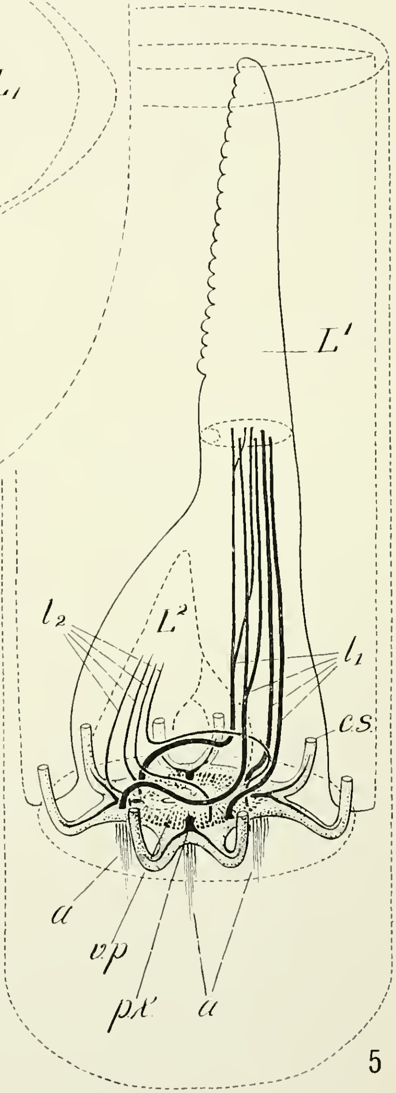

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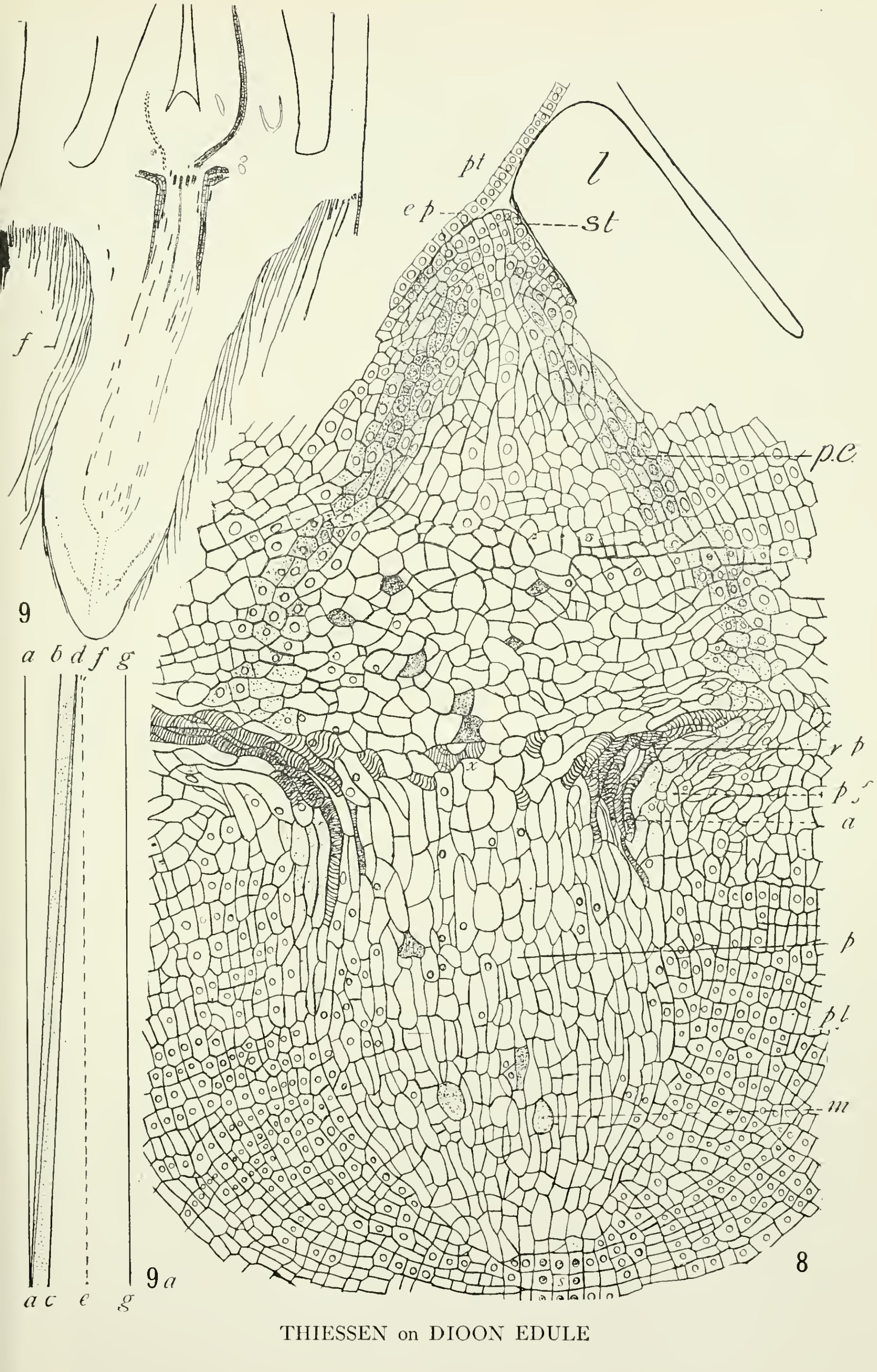

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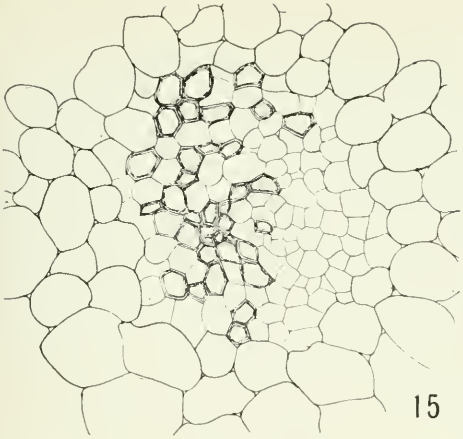
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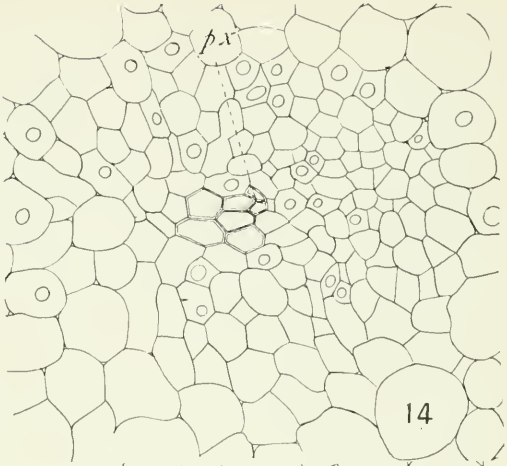

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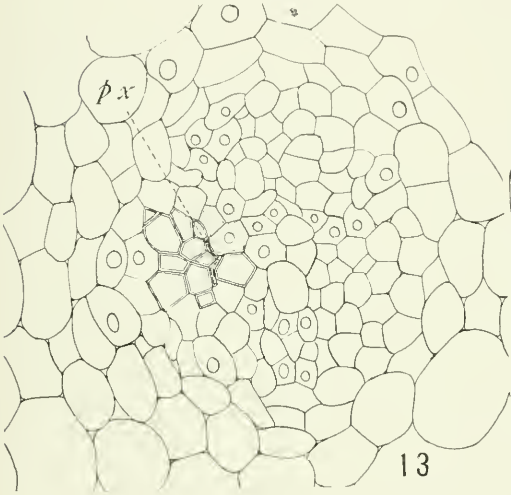

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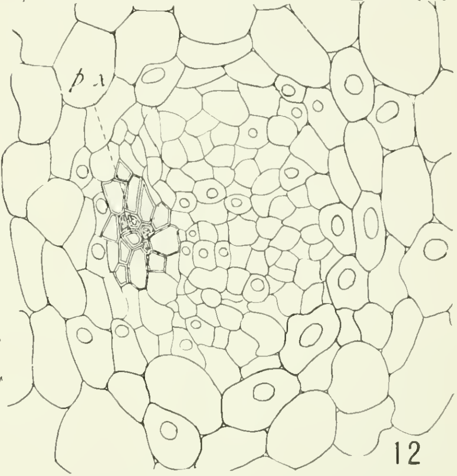

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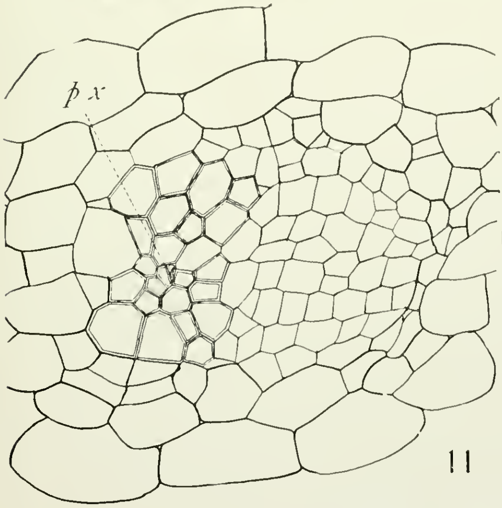

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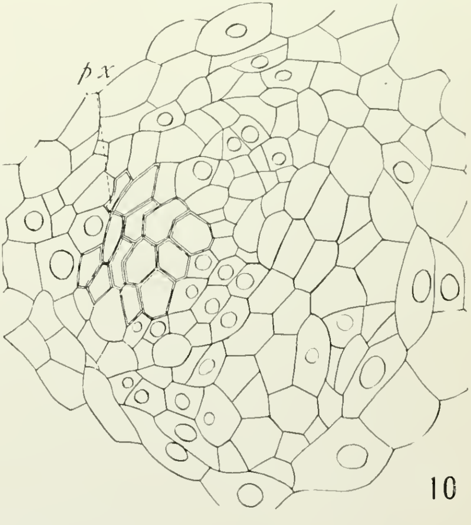

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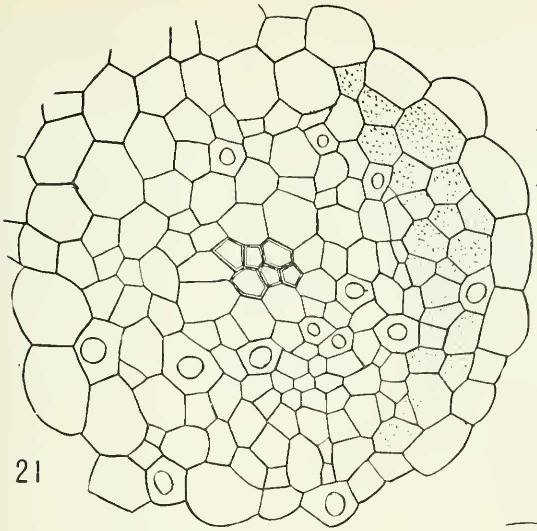
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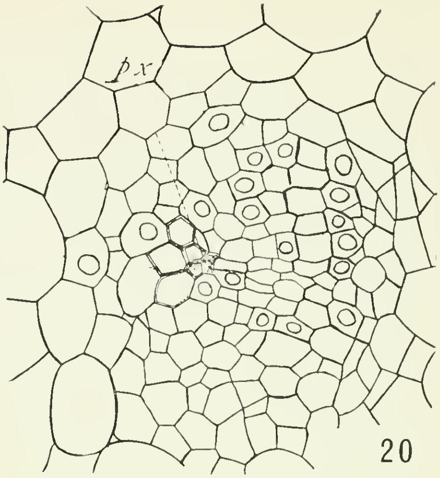

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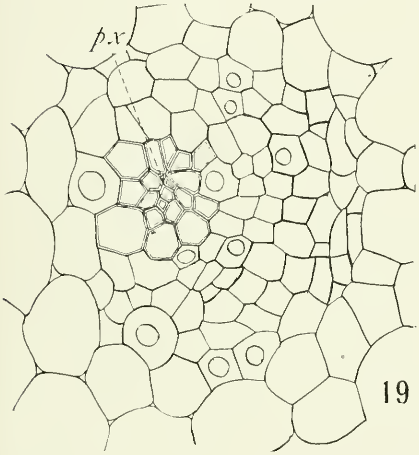

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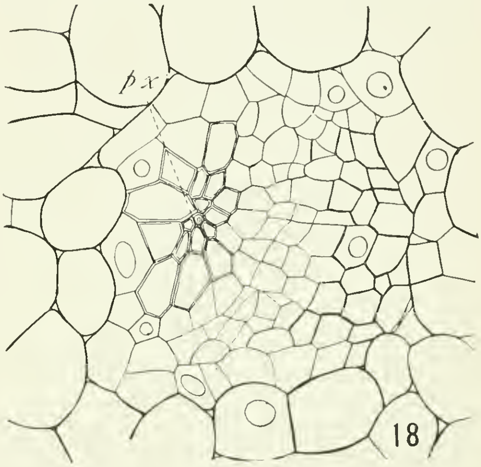

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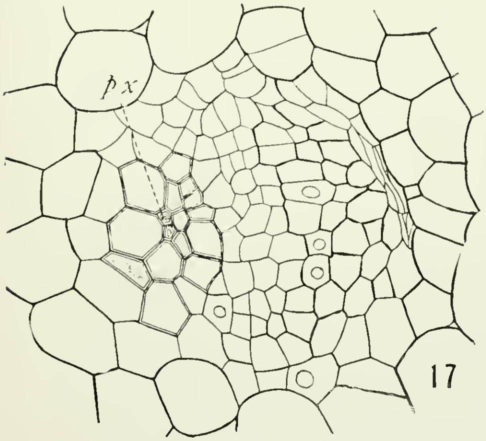

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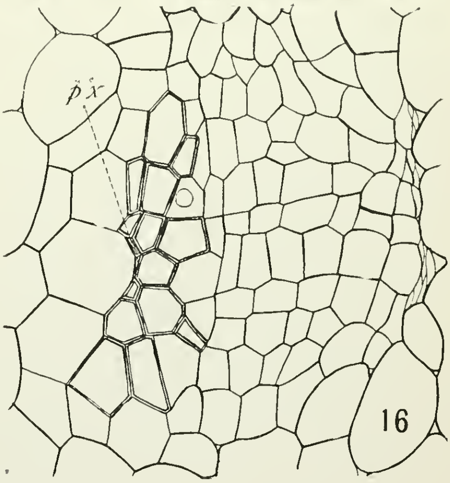

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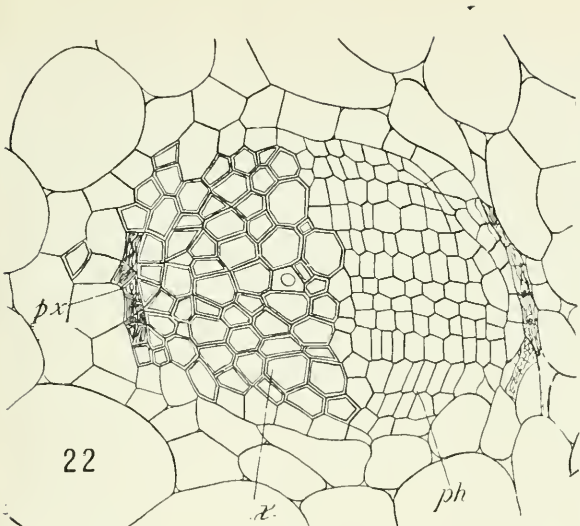

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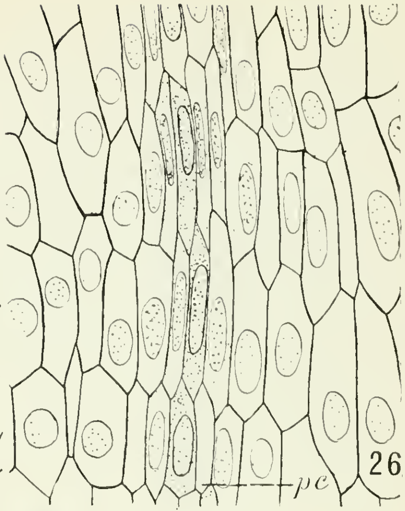

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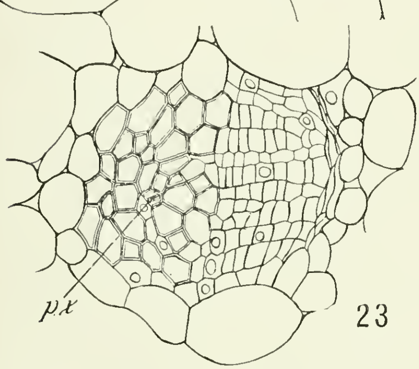

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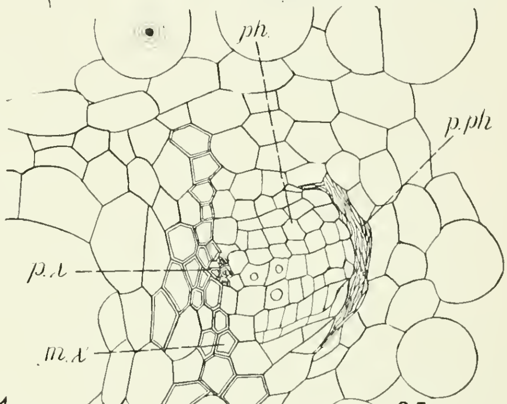

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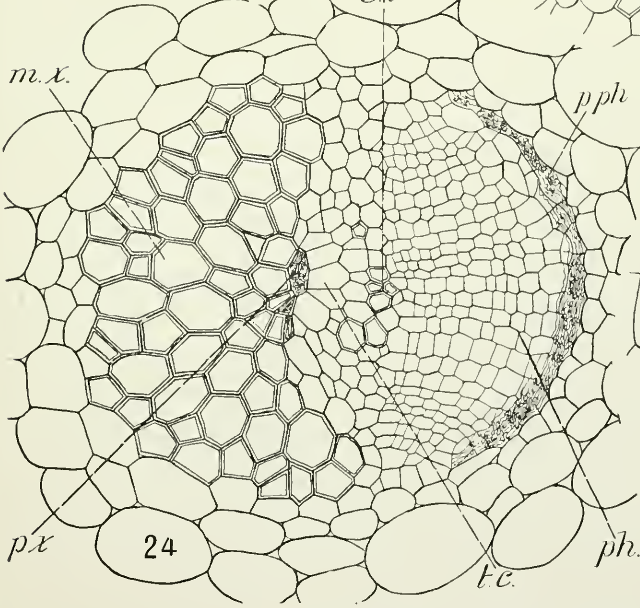

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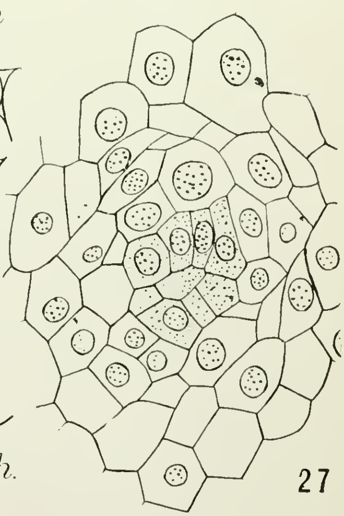

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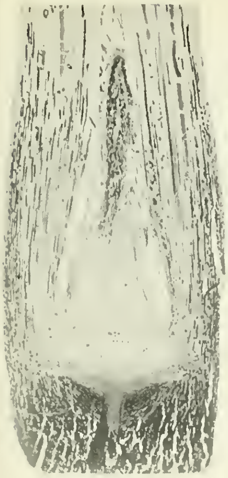

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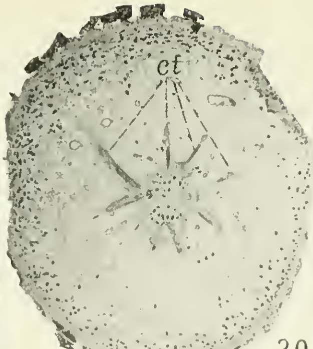

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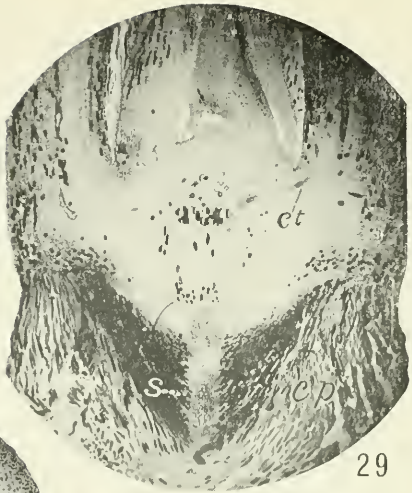

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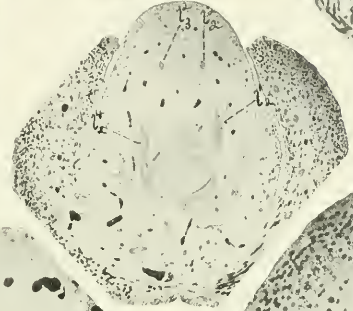

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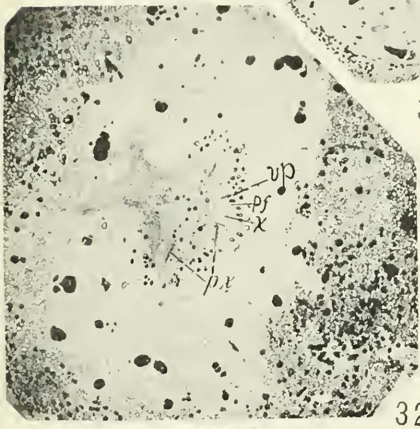

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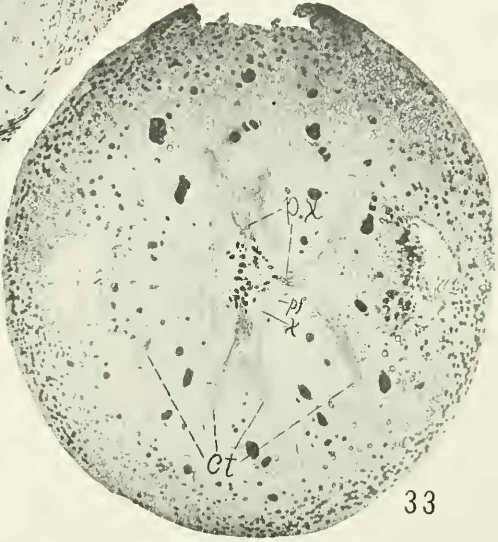

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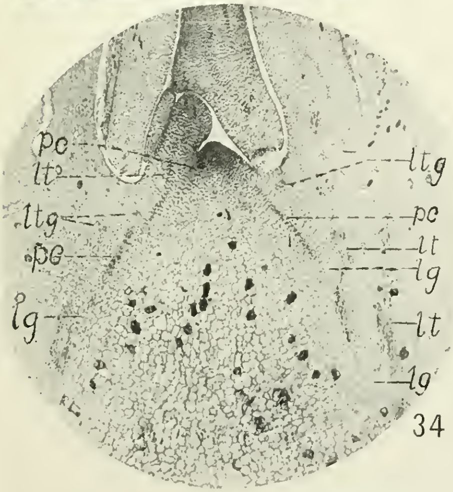

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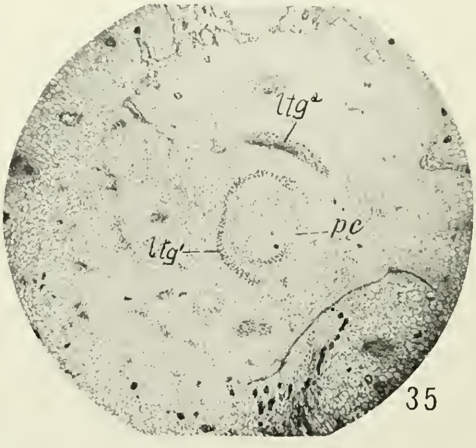

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BRYOLOGICAL PAPERS. II. THE ORIGIN OF THE CUPULE OF MARCHANTIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 120

(WITH FOURTEEN FIGURES)

CHARLES R. BARNES AND W. J. G. LAND

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II. THE ORIGIN OF THE CUPULE OF MARCHANTIA

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CHARLES R. BARNES AND W. J. G. LAND

(WITH FOURTEEN FIGURES)

The study of the cupule of Marchantia has evidently been confined to the later stages of its development, and especially to the origin of the gemmae and the order of cell division in them. Nowhere have we been able to find any account of the origin of the cupule, and the earlier stages of its development seem to have escaped observation. Its homology with other structures in the upper part of the thallus has apparently been a matter of speculation rather than of investigation. Thus, CAMPBELL casually remarks[1] that the gemma cup is apparently a specially developed air chamber, but gives no details and adduces no evidence therefor.

Although Marchantia has been much investigated and indeed has been long a favorite subject for instruction in laboratories, KNY seems to have been the first to examine any of the early stages of development of the cupule itself. This he did for the purpose of illustrating the development of Marchantia on his charts and describing the same in the accompanying text.[2] But he does not show or describe the origin of the cupule; the earliest stage referred to corresponds roughly to our *fig. 10*, when it has become a rather deep pit.

Our studies upon the origin of the air chambers in Marchantiales[3] suggested to us an inquiry into the origin of the cupule, to determine whether it could possibly be homologous with an air chamber. From an a-priori consideration of the general character of the cupule and the air chamber this idea commended itself to us both. The walls of the cup, especially the thin, lobed margin, seemed to correspond very well with the epidermal roof of the air chamber, opened wide instead of having only a narrow orifice. The gemmae, borne upon a single cell arising from the floor of the cup, might well be only a modified form of the chlorophyllose filaments of the air chamber. So natural and neat did the homology appear, that the brief prior statement of it by Campbell (*l. c.*) was discovered with a distinct sense of disappointment when we began to look into the literature. But evidence for this homology could not be found therein, and against it was to be put the fewness of the cupules, their limitation to the median line, where the air chambers are least developed, and the fact that the gemmiparous region covers many times the area of an air chamber. The matter evidently needed examination. Actual observation of the origin of the cupule speedily dissipated all

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[1] CAMPBELL, D. H., Mosses and ferns, 2d ed. 44. 1905.

[2] KNY, L., Wandtafeln. Ser. III, *pl. 84*, text p. 366.

[3] BARNES AND LAND, The origin of air chambers. BOT. GAZETTE 44:197–213. 1907.

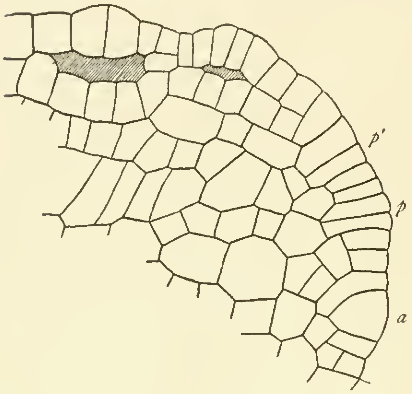

Fig. 1.—Early stage of cupule; *p*, *p'*, undivided cells, primordia of two (?) gemmiparous areas; *a*, apical cell; air chambers shaded.

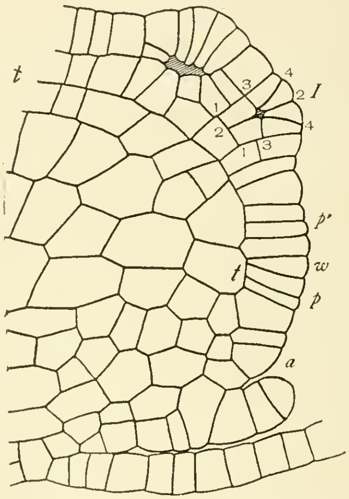

Fig. 2.—Early stage of cupule; *p*, *p'*, as in *fig. 1*; *w*, probably a rim cell between two gemmiparous areas; *a*, apical cell; *t*, *t*, line showing tissues of thallus involved in a cupule.

notions of its homology with an air chamber, as we now proceed to show.

Abundant fresh material was at hand from thrifty plants of *Marchantia polymorpha*, grown under glass by Mr. JOHN COOK, the skilful gardener of the department, who has taken much pains with the cultivation of Hepaticae. Being transferred directly from the cultures to the fixing fluids, the material was in exceptionally good condition.

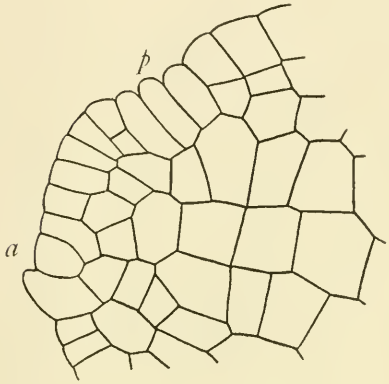

FIG. 3.—Gemmiparous area (*p*) outgrown by adjacent tissues.

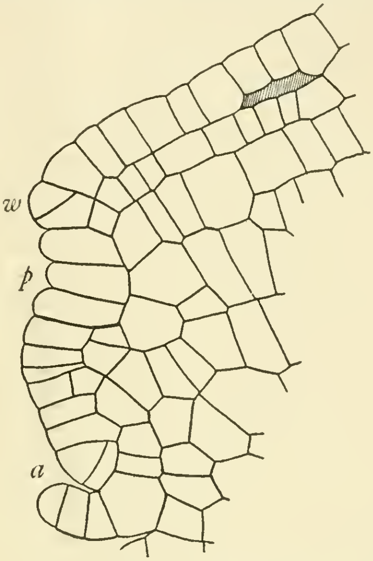

FIG. 4.—Stage nearly as in *fig. 3*; *w*, a rim cell cutting off the primordium of a lobe of the cupule.

Our figures show usually only the cell walls, the perfectly preserved contents being omitted for the sake of clearness. Mitotic figures were common, showing that the cells were in active growth. The figures are of longitudinal sections, except *fig. 13*, are all drawn to the same scale, and having been reduced one-half are now magnified about 625 diameters.

Longitudinal sections through the apex of gemmiferous plants show, as near to the apical cell as the third segment, a differentiation in the cells which are to form the gemmiparous area. Instead of dividing by several successive periclinal walls, as most of the segments promptly do, the superficial parts remain for some time conspicuously undivided (*p*, *p'*, *fig. 1*), and so are distinguishable by their depth. In particular it is the final periclinal divisions that fail. This will be more evident by following the usual segmentation of the mother cell of an air chamber. In *fig. 2*, the young air chamber *I* originated as usual in a cell which underwent one periclinal division, forming the wall *1*, *1*; then an anticlinal one (*2*, *2*); these two surface cells each divided periclinally (*3*, *3*), and cleavage occurred at the junction of walls *2* and *3*. (The curved anticlinal walls, *4*, *4*, succeeded the cleavage.) It is the divisions *1*, *1*, and *3*, *3*, or only the latter, which do not appear in the gemmiparous areas (*p*, *p'*).

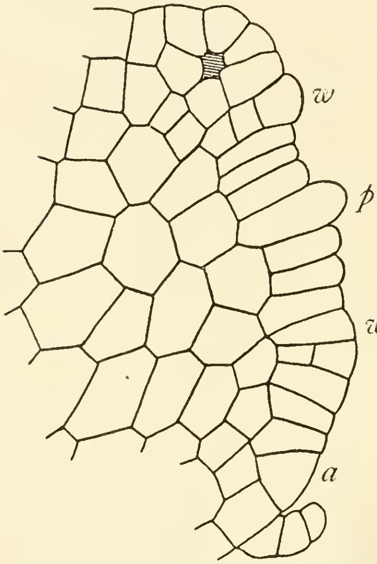

FIG. 5.—Elongation of a single gemmiparous cell (*p*); *w*, rim cells, the posterior oldest and most advanced in division.

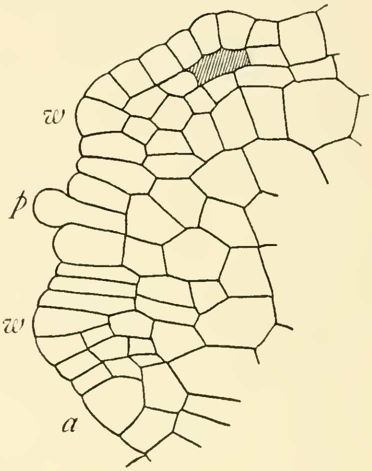

FIG. 6.—A somewhat later stage than *fig. 5*.

The relative extent of the area which these undivided cells cover, and the occurrence of somewhat different cells (*w*) between them, make it not unlikely that in *figs. 1* and *2* the primordia of two cupules are laid down in close succession; but of this we cannot be sure.

The failure of the gemmiparous cells to divide allows their neighbors to outgrow them, so that they can soon be located by the depression of the surface, as well as by their form and size (*p*, *fig. 3*). The depression, however, is not always well marked at this stage (cf. *fig. 5*). The contents, too, are sometimes distinctive, a glandular appearance being not infrequently noticeable; but as all the cells thereabouts are rich in protoplasm, this feature is not very striking.

The next step in development is the prolongation of one or more of the gemmiparous cells into papillae (*p*, *figs. 5, 6*), and simultaneously the further upgrowth of the cells at the rim of the depression, and first on the posterior margin (*w*, *figs. 4, 5, 6*). The free ends of the papillose extensions quickly enlarge (*figs. 7, 8*) and doubtless secrete some of the mucus in which the whole apical region is enveloped. Certain of the cells that form the rim divide obliquely (*w*, *figs. 4, 8*). Probably the cells thus cut off are the primordia of the thin lobes, which are so marked a feature of the mature cupule; for even in this early condition the rim becomes scalloped.

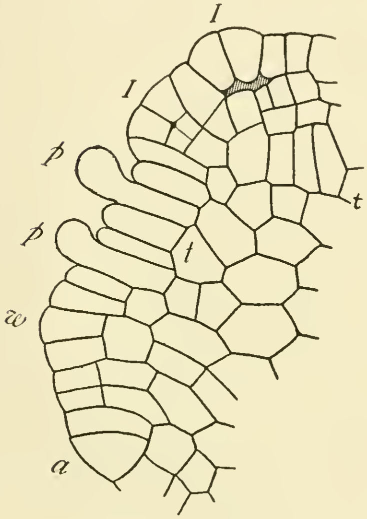

FIG. 7.—Two gemmiparous cells elongated; *I*, young air chambers; *t*, *t*, as in *fig. 2*.

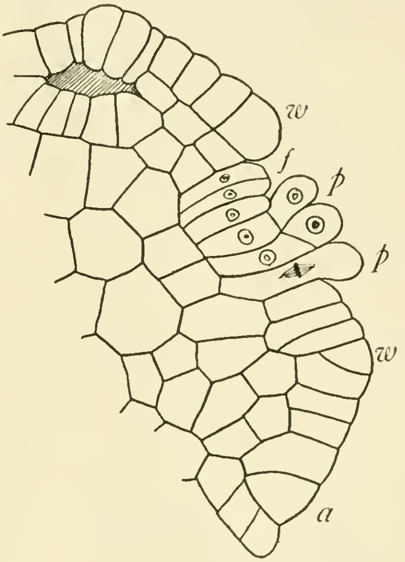

FIG. 8.—Further elongation and first division of gemmiparous cells (*p*, *p*); in *f* longitudinal division; *w*, rim cells.

After some further extension and enlargement, the gemmiparous cells divide transversely (*p*, *p*, *fig. 8*), and soon another division occurs (*p*, *fig. 9*), by which three cells are formed, a basal cell (*b*), a stalk cell (*s*), and a gemma cell (*g*). The latter continues to divide in the fashion frequently described and figured (cf. also *figs. 11, 12, 13*), and finally produces the gemma. The stalk cell undergoes no further division, but the basal cell divides longitudinally at least once (*p*, *fig. 11*). Later it may undergo repeated division, producing new

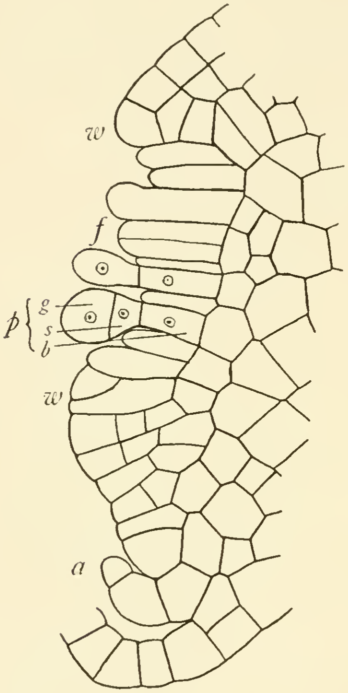

FIG. 9.—Progressing transverse (*p*) and longitudinal (*f*) division of gemmiparous cells; the former producing a basal cell (*b*), a stalk cell (*s*), and a gemma cell (*g*).

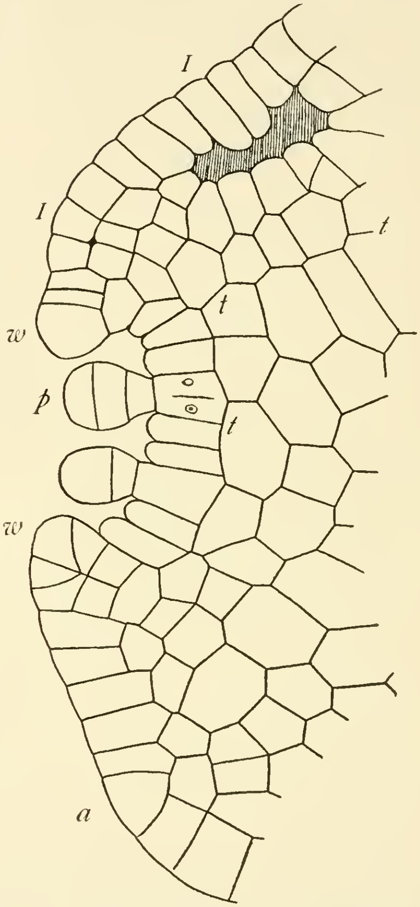

FIG. 10.—Basal cell of *p* undergoing longitudinal division; *w*, much further developed and depression deepened; *I*, young air chamber; *t*, *t*, *t*, shows relation of cupule and air chamber.

gemmiparous cells, so that each basal cell ultimately becomes the center of a group.

As the primordium of a cupule grows older, the number of gemmiparous cells forming its floor is increased by longitudinal (anticlinal) divisions (*f*, *figs. 8, 9*). The new floor cells so produced grow into papillae and soon produce gemma cells. Thus the gemmiparous area is increased in two ways: by the anticlinal division of the primordial cells, and by a similar division of basal cells that have borne or are bearing gemmae. The tissues adjacent grow rapidly, leaving the floor of the cupule soon far below the general surface (*fig. 12*), and the rim continues to outgrow the developing gemmae, which are embedded in mucus. The antero-posterior diameter of the young

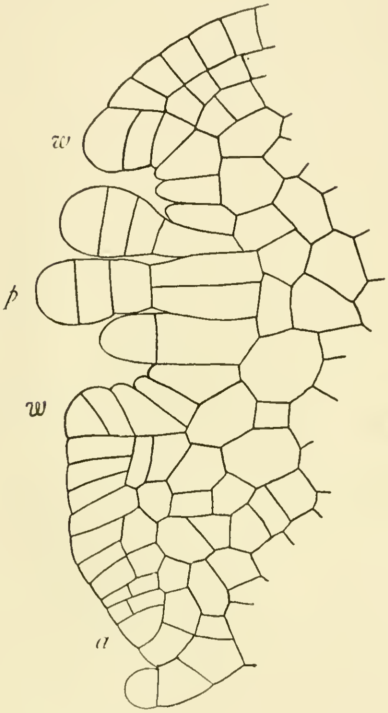

FIG. 11

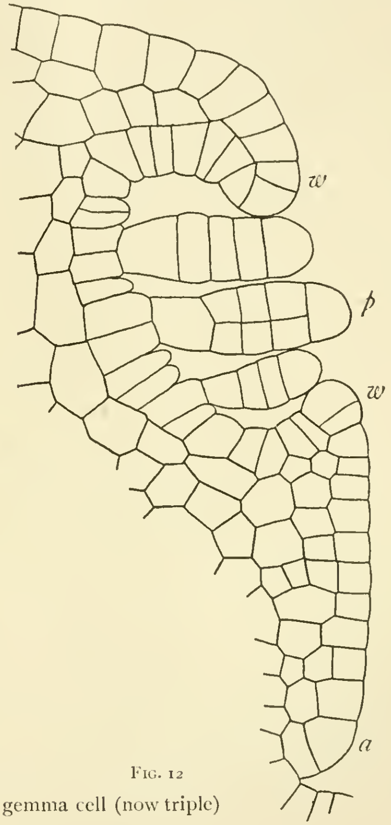

FIG. 12

FIGS. 11, 12.—Further development of gemma cell (now triple) and rim (*w*).

cupule is less than the transverse diameter, as shown by *figs. 12* and *13*, which represent respectively longitudinal and transverse sections through cupules of about the same age.

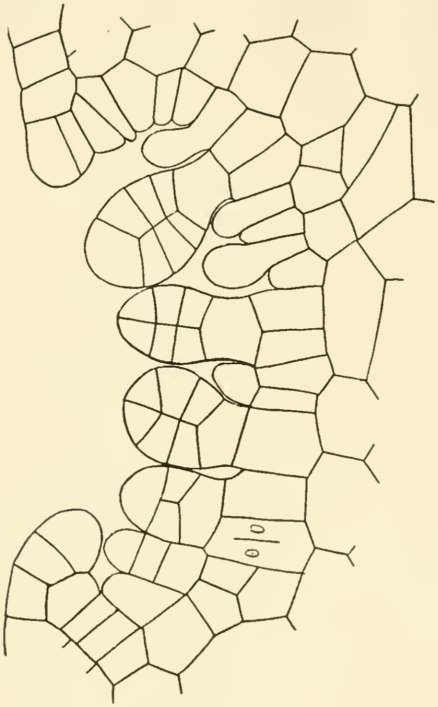

FIG. 13.—Transverse section of a cupule about the same age as *fig. 12*.

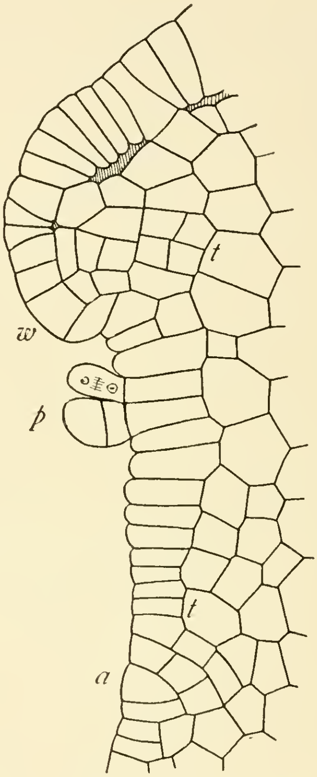

FIG. 14.—Origin of cupule of Lunularia; *p*, gemmiparous cells elongated and dividing; rim (*w*) developed only on posterior margin; *I*, young air chamber.

It is not necessary to follow the history of the cupule further, for it is a familiar object in all laboratories of instruction and has been well described.

The cupule of Lunularia has also been investigated sufficiently to show that its origin is essentially the same as that of Marchantia (*fig. 14*), except that the development of the rim takes place only on the posterior side of the gemmiparous region, which is also far more extensive. In some cases, late in development, a slight anterior elevation continues the line of the posterior rim and so suggests the circular cup of Marchantia.

The superficial origin of the gemmae is thus perfectly clear. They cannot be considered as in any sense homologous with the chlorophyllose filaments of an opened air chamber, nor has the cup any relation to the epidermal roof. The thicker part of it *contains* air chambers, and the thin part is simply a scalelike outgrowth of the epidermis. The difference between an air·chamber and a cupule becomes especially striking when a cupule originates near an air chamber, as shown in *figs. 2, 7, 10, 14*, at *I*. Then, although the gemmiparous cells are seen to be superficial, they evidently represent cells that otherwise might produce not only the roof, filaments, and floor of an air chamber, but also a considerable portion of the thallus beneath the air chamber. In *figs. 2, 7, 10*, the line, *t*, *t*, can be followed clearly, showing how deeply the gemmiparous cells involve the tissues of the thallus. It is not surprising, therefore, that the gemma cup, though of superficial origin, is a depression in the thallus, and that air chambers clothe its sides.

Incidentally we may add that the origin of the gemmiparous cells, as herein shown, precludes our acceptance of GOEBEL'S conception that in Marchantia the gemmae are homologous with "slime papillae."[5] The formation of mucus cannot be considered as a special function of any particular cells, though the so-called slime papillae have the name of "secreting" it. In fact young cells of very different origin and fate form mucus, and it is doubtful if any of the younger ones fail to form it. These "papillae" are purely superficial organs, and scarcely agree with the gemmiparous cells in anything except that at one time both project above the surface. How can the latter, which involve so considerable a part of the thallus, corresponding, as above shown, to the whole air chamber region and two or more layers of cells below it, be properly likened to such transient and superficial outgrowths as the "slime papillae"? To pronounce the two homologous throws no real light upon the nature of the gemmae, for the production of which there is such early and striking preparation.

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[5] GOEBEL, K., "Die Brutknospe von Marchantia und Lunularia kann auch als einer Schleimpapille homolog betrachten werden." Archegoniatenstudien XII. Flora 98:314. 1908.

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